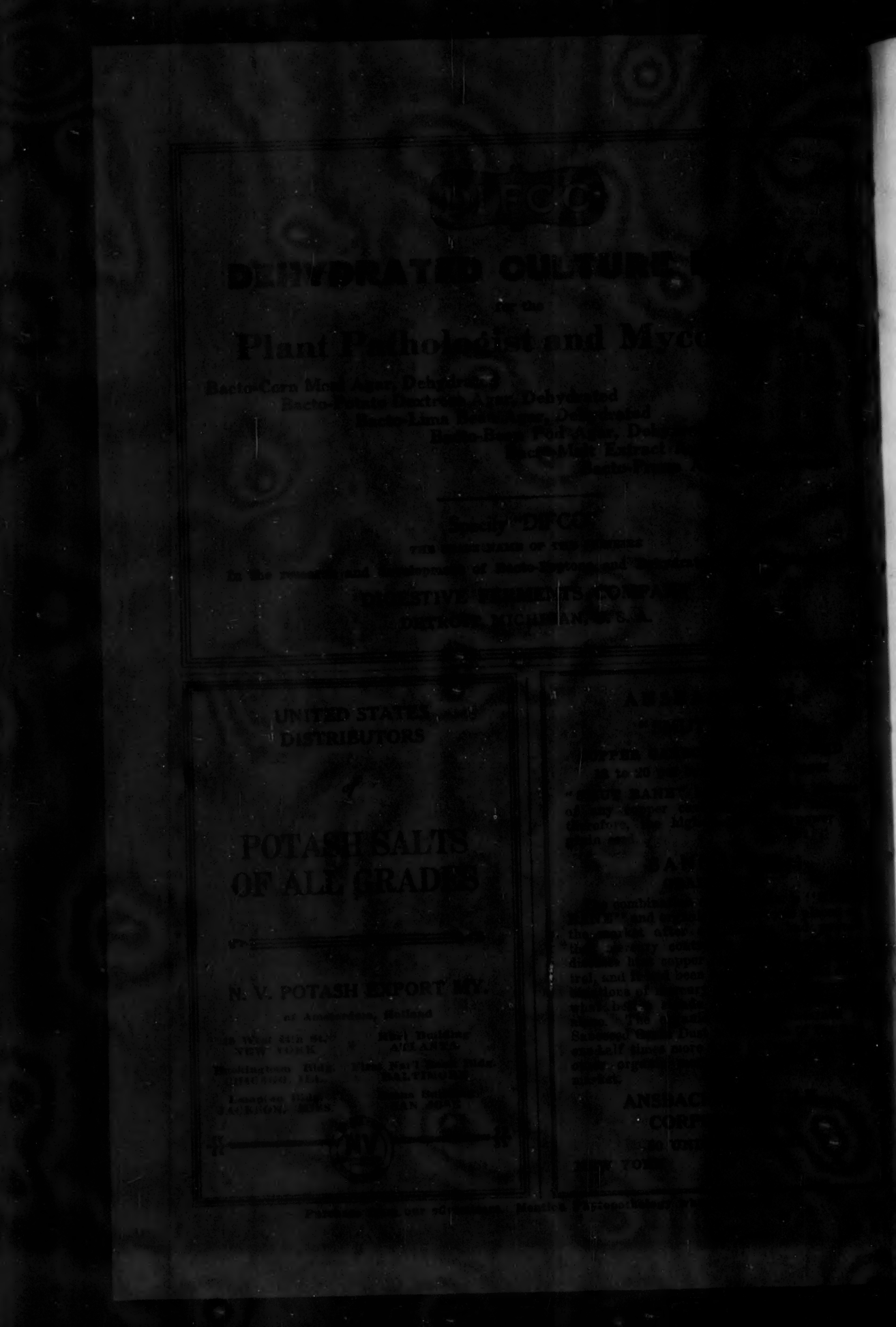

PHYTOPATHOLOGY

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THE EFFECT OF AIR TEMPERATURE ON THE PATHOGENICITY OF RHIZOCTONIA SOLANI PARASITIZING GRASSES ON PUTTING-GREEN TURF[1]

LAWRENCE S. DICKINSON

INTRODUCTION

The observing green keeper recognizes a correlation between weather conditions and the appearance of the disease known as large brown patch. He frequently speaks of "brown patch weather." Little or no practical use, however, is made of these observations. The active cause (*Rhizoctonia solani* Kühn) of the disease is known, but little consideration has been given to air temperature as a possible factor in its control.

Rhizoctonia solani is the fungus which causes the disease commonly called large brown patch. It attacks most grasses and is particularly injurious to putting-green turf. It develops a fine white cobweb-like mass of mycelium on or within the turf. The hyphae of this mycelium enter the grass leaf (usually through the stomata) and break down the cells, causing the leaf to shrivel and become brown. Only in the very severest attacks do the hyphae enter the grass stems or injure the grass roots. The fungus forms sclerotia, or resting bodies, that are able to withstand unfavorable conditions. These sclerotia are found in most cultivated soils as dark brown, membranous, persistent bodies of corky texture and visible to the naked eye. They are about 2 mm. in diameter or larger. During weather unfavorable to the development of the fungus, sclerotia may fall upon the ground or remain on the grass leaves and stems until the return of conditions favorable to their growth. They are hardy to the usual extremes of temperature and, during the winter, are preserved in the soil or upon the dormant grass leaves and stems. The mechanical destruction of the sclerotia is impracticable, and their corky texture makes them resistant to fungicides.

Scientists (2, 4, 5, 7, 11) have shown that the ability to induce certain parasitic fungous diseases is conditioned on a specific range of soil temperature, together with other factors, and that there are maximum, optimum,

[1] Massachusetts Agricultural Experiment Station Contribution No. 105, 1930.

and minimum temperatures for the development of a given fungus. Jones (3) and Richards (9) found that Rhizoctonia is most virulent to potatoes at soil temperature of 15°–21° C. "with a distinct and rapid lessening of the diseased condition at higher temperatures" up to 24° C., above which development was only of minor significance. They also found that the sclerotia at a soil temperature of 15°–18° C. produced the greatest number of hyphae. On the other hand, Rolfs (10) and Peltier (8) found that high temperature (30° C.), together with too much or too little moisture, determined to a large degree the virulence of various strains of Rhizoctonia. Monteith (6) demonstrated that Rhizoctonia develops best at 83° F. when cultured in petri dishes stored in constant-temperature chambers. However, almost no growth showed at 50° F. or 61° F. and only a third as much at 94° F.

A review of the literature previously cited showed that nearly all scientific studies on Rhizoctonia have been made with potatoes as a host and that the temperature studies have been made with the fungus growing on potatoes in soils kept at various constant temperatures or by culturing the fungus on a nutrient medium in petri dishes stored at various constant temperatures. It is to be noted that none of the investigators has reported on the appearance of large brown patch or the development of the mycelia of Rhizoctonia in their relation to changing air temperatures.

As the sclerotia and mycelia of Rhizoctonia generally are prevalent in most soils and large brown patch appears only occasionally, it would seem that the disease is not so much dependent upon the presence of sclerotia or mycelium as it is on favorable environmental conditions.

In turf the sclerotia are subjected more to air than to soil temperatures, since most of them are on the surface of the soil or on parts of the grass. This surface soil of short-grass putting-greens is more quickly affected by a change in atmospheric temperature than surface soil under taller grasses of lawn turfs. Furthermore, it has been observed by agrostologists, pathologists, and green keepers that putting-greens are more susceptible to brown patch than lawns or fairways. On putting-greens the mycelium frequently appears at the greens edge and not in the adjacent taller grass of the same species. Thus, it would seem, the sclerotia develop mycelia more favorably where the atmospheric temperatures exert their greatest influence.

OBSERVATIONS LEADING TO LABORATORY STUDIES

During the past five years the writer has obtained many data concerning these environmental conditions which favor the appearance and development of the large-brown-patch disease. The sources of these data were personal observations, contact with green keepers, and professional journals.

SUMMARY OF DATA FROM GREEN KEEPERS AND PROFESSIONAL JOURNALS

Because the reported observations have been made in the morning after development of large brown patch had started, investigators and green keepers have been led to believe that the disease is nocturnal (6, 1). On the other hand, in the test plots at the Massachusetts Agricultural College, it has appeared in both mid-afternoon and late afternoon, following certain environmental conditions. Also, it is quite probable that this disease, appearing on many greens in the late afternoon, was not observed until the following morning.

The large brown patch may appear on any putting-green comprising grasses that are susceptible to the disease, but it appears definitely and more often on putting-greens where the soil is low and poor and the air drainage is defective. The disease also appears following afternoon watering on hot days and subsequent to a rapid drop in air temperature from 80°–95° F. to 60°–70° F. Putting-greens that have been rapidly cooled by a chilling wind, rain, or the settling of cool air over low areas are very frequently attacked by the disease.

The disease appears as a mild attack or fails to appear during cool periods, continued hot periods, following evening watering or dewless nights, or on putting-greens that receive little or no artificial watering.

Humidity appears to influence the appearance of the disease only as it influences the air and surface soil temperature. Under certain temperature conditions the disease will appear on upland and exposed putting-greens, as contrasted to the lowland and enclosed greens.

FIELD OBSERVATIONS

Three experimental putting-greens at the Massachusetts Agricultural College are being employed in this investigation. The greens are located side by side, and each one has a uniform soil of medium heavy loam. The greens are exposed to direct sunlight until late afternoon when the western halves are in shadow. Air circulation is unrestricted except for a distant wind-break on the south and east sides. Each green is seeded or planted in ten-foot squares with varieties and strains of putting-green grasses. (Metropolitan, Washington, Flossmore, Virginia, Columbia, and Rockford strains of creeping bent; Kernwood, Newport, and Fall River strains of velvet bent; Rhode Island, Colonial, South German, Cocoos, and Fiorin bent; Red and Chewings fescue.) The mean elevation above sea-level is 242 feet. The areas are Green I—1,000 square feet, Green II—4,200 square feet, Green III—3,000 square feet.

Three years ago it was noted that the appearance of large brown patch regularly followed a rapid fall of air temperature, provided the cooling

was immediately followed by a rise in air temperature. It also was noted that the fungus appeared from three to three and a half hours after the rise in air temperature began, and, if its growth were destroyed at once by attrition, such as poling, by vigorously sweeping a bamboo pole back and forth on the surface of the green, or by fungicides, there was no injury to the turf. It was observed also that the growth of the mycelium was more rapid when the cooling was accompanied by rain. The practical significance of such conditions was realized and led to further studies during the past two years. The environmental conditions have been so accordant that each appearance of the disease and its degree of severity could be forecast.

Definite Data of Air Temperature as Correlated with Appearance of Large Brown Patch. To obtain temperature data, a Weather-Bureau instrument shelter was placed in the center of the putting-green area. In the shelter were installed a standard thermograph and corrected maximum and minimum thermometers. The thermograph is checked twice daily against the thermometers and is one foot above the turf. The thermometers are one foot higher. In presenting these data it is realized that the air temperature outside the shelter has a wider range than on the inside.

The humidity records were obtained during 1928 by means of a sling psychrometer rotated breast-high above the grass. Because the humidity records were inconsistent at the time of the appearance of the disease and because of the greater degree of humidity in the dew-laden grass little value could be given to the humidity readings. They were not recorded in 1929. Further meteorological data, such as sunshine, cloudiness, and rainfall, are obtained from the college station, located five hundred yards to the west of the experiment greens.

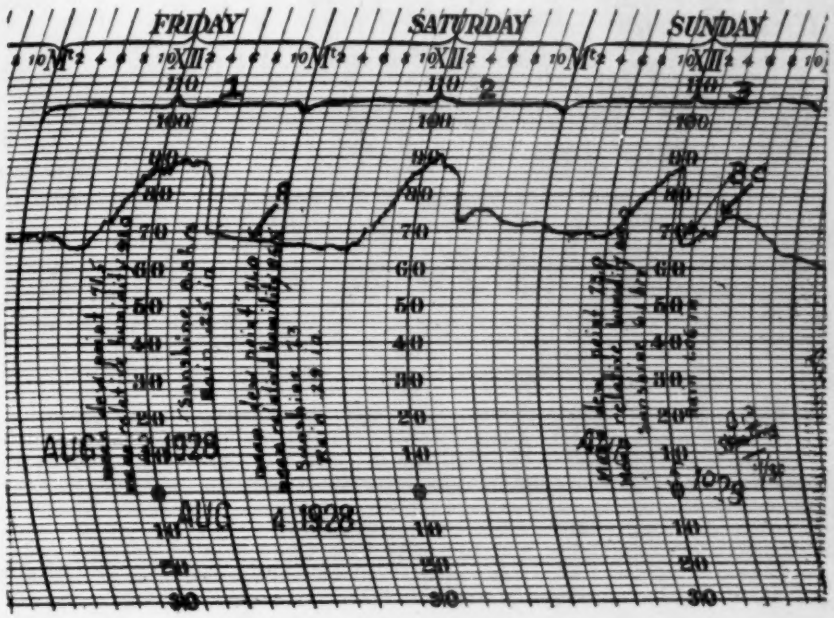
The records also showed that if the optimum temperature range (64°–68° F.) is reached from a lower temperature, the air must remain from eight to ten hours at a nearly constant temperature within the optimum range to secure growth of sclerotia.

Summary of Field Observations. A summary of the evidence secured from field observations to date clearly indicates a close correlation between air temperature and the appearance and development of *Rhizoctonia solani*. Two phenomena must be given consideration, the growth of short mycelia from the sclerotia and the development of these mycelia from inactive organisms dependent on the sclerotia for food to independent parasitic organisms. (Field and laboratory observations repeatedly showed that the sclerotia grew short mycelia (10–15 mm.) soon after they had been chilled. These short mycelia remained inactive until environmental conditions favored their further development or they were destroyed.)

Development of Mycelia from Dormant Sclerotia

Representative Thermograph Readings Selected from Three Seasons' Records

Record A



Under field conditions, if the optimum temperature was reached from a higher temperature, growth did not occur unless the change was very rapid.

1. No growth of sclerotia was expected, since abrupt drop in temperature failed to reach the optimum range (64°–68° F.) until four hours after the fall, indicated as A.
2. No growth of sclerotia was expected, since the optimum range was not reached.
3. Very severe attack was expected and did occur, since the sudden drop in air temperature was to the optimum range, indicated at B. Spread of mycelium began at 4:30 p. m. (73° F.), indicated at C. Immediate poling completely checked the mycelium already developed. On a nonpoled plot the spread of mycelium was noted until the temperature fell to 70° F.

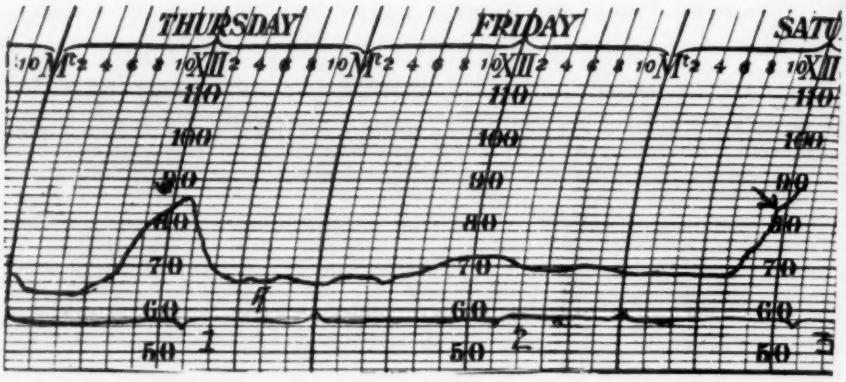
The optimum temperature range for the growth of the short mycelia from the sclerotia appears to be between 64° and 68° F., and the required duration of temperature within such range is variable.

The mycelia will appear in from 30 minutes to one hour after the chilling to the optimum stage.

The development of the fungus to the point of becoming actively parasitic is concurrent with a rise in air temperature.

Virulence appears to require a rather rapid rise in air temperature from 64°–68° F. to between 80°–85° F. Parasitism commences at about 73° F. and ceases at 90° F. If the air temperature falls below 62° F. the mycelia on the sclerotia are destroyed.

Record B



After short growth from the sclerotia the mycelia will remain inactive until environmental conditions favor their development, or they may be destroyed by attrition or excessive heat or cold.

1. It is to be noted in the instance illustrated that on Thursday growth of the sclerotia should be expected at between 7 and 8 p. m. (A). The temperature was too low for rapid mycelial development but, realizing that there were many sclerotia covered with very short mycelia, the green was not disturbed.
2. On Friday no attack was noticeable to the ordinary observer because the mycelia had not developed sufficiently to be visible as cobweb-like masses.
3. Saturday observations at 7, 8, 9, 10 and 11 a. m. found no apparent attack of the fungus. Observation at 11:45 a. m. found that the mycelia had developed so rapidly that the usual circular diseased patches were very noticeable.

Following the occurrence of short mycelial growth from the sclerotia further development of the organism probably is dependent on high humidity as well as air temperature.

No reference has been made to other recorded meteorological data, such as rainfall and sunshine, because they failed to show vital significance to the mycelial development except as they affected the air temperature. The mycelium develops from the short nonparasitic stage more rapidly on moist grass than on dry, and it makes little difference whether the moisture on the grass is obtained as dew, rainfall, or artificial watering. Yet, several attacks have been observed on dry grass.

The observations recorded are practical evidence of the soundness of the opinion that a rapid chilling of the air temperature is a very important factor in the appearance of *Rhizoctonia solani* on grasses. In the appearance of the disease, moisture seems to be of more consequence as a modifier of the air temperature than as a direct factor in the growth of the sclerotia.

It is to be noted these records showed that during the "brown patch season" there were only 5 per cent. of the days in which environmental conditions favored growth of the sclerotia and subsequent mycelial development. By being able to forecast the appearance of the disease and confine

TABLE 1.—*Records of field observation after sufficient data had been collected to indicate favorable and unfavorable conditions*

	1928	1929
Date of first record	July 1	May 27
Date of last record	Oct. 31	Oct. 1
Number of days observed	123	123
Number of days favorable to virulent attack	7	5
Number of attacks occurring on days unfavorable to virulent attacks	0	0
Number of days disease observed	7	5
First attack	July 9	June 25
Last attack	Aug. 17	Aug. 22

the use of fungicides to such times, a green keeper can effect a considerable saving in the cost of golf-course maintenance.

Practical Adaptation of Observations. To test the practical adaptation of the observations previously reported, two methods of control have been used: (1) Fungicides as preventives and controls and (2) attrition by severe poling. Nontreated and nonpoled plots were used as checks. The results when summarized show:

1. A fungicide, if applied only when environmental conditions indicate its need, is inexpensive, a complete protection from attack, and seldom injurious to the turf.

2. A fungicide as a control, if applied very soon after the sclerotia produce the short mycelia, prevents further development of the mycelia, is inexpensive, and is seldom injurious to the turf.

3. Attrition by poling very early each morning (a few hours after sunrise) destroys the activity of mycelium that may have developed during the preceding 24 hours. Control by daily poling has been 60 per cent effective on the experimental plots. Complete control by this method is not possible because favorable environmental conditions for the growth of the sclerotia occasionally occur in late morning or during the afternoon, a fact overlooked by the majority of green keepers.

4. Attrition by thorough poling soon after the sclerotia germinate affords approximately 100 per cent control, is inexpensive, and causes only a roughing of the turf.

PETRI-DISH EXPERIMENTS[2]

Preliminary Laboratory Experiments. The object of the laboratory experiments was to check the field results by placing the sclerotia under more or less controlled conditions.

[2] These experiments were performed at the Boyce Thompson Institute for Plant Research, Inc., at Yonkers, New York. To Dr. William Crocker, Director of the In-

A medium was prepared by placing a small amount of corn meal in each of six Erlenmeyer flasks and covering it with distilled water. The flasks were then plugged with cotton and sterilized in an autoclave.

Rhizoctonia solani sclerotia and mycelia were transferred to the Erlenmeyer flasks, the transfer being made in a sterilized transfer case. The sclerotia developed mycelia which, in turn, formed sufficient sclerotia to be used as supply colonies for future experiments.

In each series of experiments an equal amount of potato-dextrose agar was placed in each petri dish as a medium. All transfers of Rhizoctonia sclerotia were made with a sterilized needle and in each colony there was placed approximately the same number of sclerotia.

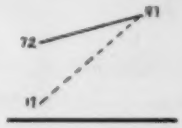
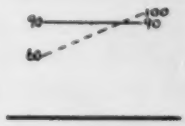
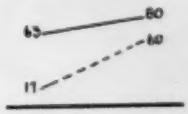
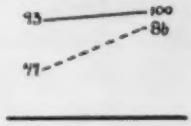
Measurements of mycelial growth were obtained by holding the petri dishes to a strong light and carefully tracing the outline of the mycelial area on cross-section paper divided 10 x 10 to the half inch. Thus, each unit of measurement represents 1/400 of a square inch.

TABLE 2.—*Table showing average mycelial growth in units from one colony of sclerotia in petri-dish cultures*

No. of exp.	Conditions to which sclerotia were subjected	Sclerotia chilled to optimum temperature range		Sclerotia not chilled to optimum temperature range (Checks)	
		Hours after chilling period		Hours after chilling period	
		3½	17½	3½	17½
I.	Average mycelial growth of all sclerotia used in the experiment	24.3	438	6.3	225.8
II.	Maximum temperature 30° C. Minimum temperature 25° C. (Except chilling period)	25.8	449.8	10.3	347.3
III.	Minimum temperature above 30° C. (Except chilling period)	22.8	364.5	11.2	319.8
IV.	Maximum temperature below 25° C. (Except chilling period)	0	40.5	0	35
V.	Maximum temperature 35° C. Minimum temperature 25° C. (Except chilling period)	21.1	480.2	10.2	411.3
VI.	Minimum temperature above 35° C. (Except chilling period)	13.75	43.75	0	16

stitute, and his associates, the writer expresses sincere appreciation for their helpful advice and criticisms; also, for the use of the equipment at the Institute. Acknowledgment is also made for the valuable assistance rendered by Dr. William H. Davis of the Department of Botany at the Massachusetts Agricultural College.

Summary graph of petri-dish experiments with Rhizoctonia solani

No. of exp.	Conditions to which sclerotia were subjected	Percentage of sclerotia[3] showing mycelial growth	
		3½ hrs. after chilling	17½ hrs. after chilling
I.	Sclerotia chilled to optimum temperature range and warmed above prechilling temperature		
II.	Sclerotia chilled to optimum temperature range and warmed to prechilling temperature		
III.	Sclerotia chilled to optimum temperature range and warmed to a lower temperature than that before chilling		
IV.	Sclerotia chilled to optimum range but warmed to a maximum temperature above 30° C. either before or after chilling		
V.	Sclerotia chilled to optimum temperature range but warmed to a maximum temperature of 30° C. either before or after chilling		
VI.	Sclerotia chilled to optimum temperature range but warmed to a maximum temperature of 25° C. either before or after chilling		

[3] ———— Sclerotia chilled to optimum range (17°–20° C.)
------ Sclerotia not chilled to optimum range (checks).

The examination of the dishes was made at room temperature and the notes taken as rapidly as was consistent with accuracy. The air-temperature changes were obtained by placing the dishes in standard temperature chambers regulated to the desired temperatures. (19°, 20°, 25°, 30°, 35°, 30° C.)

At intervals all dishes were placed in the 30° C. chamber to provide a most favorable growing condition.

Table 2 indicates the following: (1) Twenty degrees C. appears to be too cool for vigorous mycelial development. (2) Mycelial development is retarded by a rise in air temperature to 35° C. or above. Total mycelial growth from sclerotia at start of experiment, 1,229 units. Total growth after having been warmed to 35° and 39° C., 966 units, showing a decrease of 29 per cent. (3) Chilling appears to be very essential for the growth of sclerotia if the air temperature is warmer than 35° C. (4) Mycelial growth in all cases was very weak at the time of the first observation and could be destroyed very easily by fungicide or attrition.

POT EXPERIMENTS

Growth of Sclerotia on Earth and Grass. The object of this experiment was to obtain data as to the growth of the sclerotia of *Rhizoctonia solani* when placed in field conditions but subjected to temperature control.

Cultures of Metropolitan creeping bent, in two-inch flower pots, were used for the experiments on turf, and loam in two-inch pots was used for the experiments on earth. Small flower pots were necessary because the temperature chambers were small.

In each series of experiments approximately equal numbers of sclerotia were transferred from a culture in an Erlenmeyer flask to the grass or soil. The transfers were made with a sterilized needle and the sclerotia placed on the surface of the loam and on the grass about one-half inch above the earth. Pots were marked: 1, *grass damp;* 2, *grass dry;* 3, *earth damp;* 4, *earth dry.* Four pots, one of each number, formed a series, and all temperature changes were made with series and not with individual pots.

In each experiment all pots were kept three hours in an equalizing or starting temperature, just as in the case of petri-dish experiments. Those scheduled for chilling were taken directly from the first chamber to the cool chamber (18°—19° C.) and kept there from 30 to 45 minutes and then placed in warmer chambers as scheduled and held there from 14 to 15 hours.

Observations were made at each changing of chambers and at the end of the 14-hour period. Measurement of growth was made on a comparative basis. The pot showing the greatest development in each experiment was credited with 20 points, credit to the other pots being in proportion to their relative development. Growth of the sclerotia was recorded by actual count of colonies showing any mycelial growth. At the close of each experiment complete control of the disease was effected by the mechanical disturbance of the mycelia.

Summary of the Pot Experiments. 1. The growth of the sclerotia was hastened and increased if the sclerotia were cooled to the optimum range (64°—68° F.) (17.5°—20° C.) for 45 minutes.

2. Sixty per cent of all sclerotia that were chilled showed short mycelial growth at the end of the chilling period.

3. No sclerotia kept at a constant temperature showed mycelial growth at a time corresponding to the end of the chilling period.

4. Mycelial development was greatly increased in those instances where the sclerotia had been chilled.

5. Chilling to the optimum temperature range appears necessary to the growth of the sclerotia under practical conditions, because normal air temperatures do not remain constant at 25° C. or 30° C. (77° or 86° F.) long enough to promote the growth of the sclerotia.

TABLE 3.—*Record showing growth of sclerotia and development of mycelia under damp and dry conditions*

Pots	Percentage of sclerotia showing mycelial growth	Comparative mycelial development
Grass damp	68	27
Grass dry	53	18
Earth damp	83	45
Earth dry	71	41
All grass	60	22
All earth	77	42
All damp	76	35
All dry	63	30

The results shown in the above table are similar to those noted in field observations, namely:

1. Moisture is favorable to the growth of the sclerotia and development of the mycelia but is not of vital significance.

2. Sclerotia grow better and the mycelial development is greater when the sclerotia are on the earth than when they are on the grass.

The laboratory results, though not entirely consistent, indicate that there is a scientific value in the field observations that a rapid chilling of the air temperature is a very important factor in the appearance of *Rhizoctonia solani* on grasses and that further scientific investigation is warranted.

PRACTICAL SIGNIFICANCE

The practical siginficance of this investigation is that it indicates:

1. Favorable air temperature is vital to the growth of the sclerotia of *Rhizoctonia solani.*

2. There are two phenomena to be considered: The growth of short mycelia from the sclerotia and the development of the mycelia after such growth takes place.

3. That it is possible to forecast accurately the appearance of the fungus, and therefore limit control measures to such times, thus effecting a great saving in cost.

4. It is practicable to check effectively the development of the mycelia by mechanical attrition, and, if attrition is applied soon after growth of the sclerotia takes place, control is as complete as when fungicides are used and with less injury to the turf

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AN ABERRANT PHYSIOLOGIC FORM OF PUCCINIA TRITICINA ERIKS.[1]

C. O. JOHNSTON[2]

INTRODUCTION

The recent demonstration by Newton and Johnson[3] of the occurrence of mutation in *Puccinia graminis* Pers. is of great interest and importance to the cereal-rust investigator. It gives material weight to an important theory on the origin of physiologic forms, although the mutant types observed by them did not differ from the parental types in their infective capabilities. The mere establishment of the fact of mutation under controlled conditions is of great importance even though the mutants apparently were not new physiologic forms. It seems reasonable to suppose that if the rust fungus can mutate for such a heritable character as color, it also can mutate for the equally heritable character of physiologic specialization. It seems, therefore, that in mutation we may find a possible origin of physiologic forms, as well as in the phenomenon of heterothallism, demonstrated by the classic experiments of Craigie.[4, 5, 6]

The mutations observed by Newton and Johnson[7] occurred in *Puccinia graminis tritici* and involved the characters of color, viability of urediniospores, and spore dimensions. Similar mutations have not been described in the other cereal rusts. Since there is, however, a strikingly close analogy between these fungi in morphological and physiological characters, we may expect to encounter them in the course of future experiments. In the writer's experiments with the leaf rust of wheat, *Puccinia triticina*, mutation has not been observed to occur under controlled conditions. An aberrant form has been studied, however, which may have arisen through mutation. It was particularly interesting in that it differed from other known forms, principally in length of incubation period and in spore color.

[1] Contribution No. 266 from the Department of Botany and Plant Pathology, Kansas State Agricultural College, Manhattan, Kansas, in cooperation with the Office of Cereal Crops and Diseases, U. S. Dept. of Agriculture.

[2] Associate Pathologist, Office of Cereal Crops and Diseases, U. S. Dept. of Agriculture.

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COMPARATIVE GREENHOUSE STUDIES

Greenhouse studies have been made at Manhattan, Kansas, for four years on physiologic specialization in *Puccinia triticina*. More than 200 collections of leaf rust have been cultured during the progress of these studies. Many physiologic forms have been found that differed in their ability to infect certain varieties of wheat, but all save one were almost identical in color, length of incubation, and certain morphologic characters.

In June, 1927, a collection of leaf rust was received from Mr. P. B. Dunkle, superintendent of Texas Substation No. 6, at Denton, Texas, which has proved to be markedly different from other known forms of *Puccinia triticina* in several characteristics. The rust was collected by Mr. Dunkle because it occurred on Texas No. 3015–63, a pure-line selection of Mediterranean wheat known to be highly resistant to leaf rust. The writer had visited the Denton station on May 10 and at that time the upper leaves of Texas No. 3015–63 were practically rust-free, although they exhibited marked flecking. Nearly two weeks later, on May 23, Mr. Dunkle noted the presence of numerous small, light-color uredinia on that variety and sent specimens to Manhattan for examination.

The rust, upon receipt at Manhattan, was given the culture number 199 and transferred to seedlings of Kanred, C. I. 5146, then being used as a susceptible variety upon which collections of leaf rust were cultured and increased in quantity. One week later there were no signs of infection having taken place, but the inoculated seedlings were not discarded. At the end of ten days a few flecks had appeared, but no uredinia had developed. This was an interesting situation in view of the fact that a number of other collections being cultured at the same time were showing flecks at the end of 4 to 6 days and developing uredinia in 8 to 10 days. In fact, most cultures of leaf rust of wheat then under investigation were fully developed at the end of 12 days. In the case of culture No. 199, however, no uredinia appeared until the 15th day after inoculation, and full development was not reached until much later.

Since several other cultures of leaf rust were being grown simultaneously in the greenhouse with culture No. 199, it was deemed advisable to repeat the studies under very carefully controlled conditions. The original observations were made on a culture grown in a cloth cage. These cages were made of a good grade of muslin stretched over a frame one foot square and a foot tall, the top being covered by a pane of glass. Such cages have been used in greenhouse rust studies at Manhattan and have proved to be very effective in preventing mixtures of cultures and forms.

When culture No. 199 was transferred for further study, however, the transfers were made from 6 uredinia taken at random from the original

culture to wheat seedlings grown under aseptic conditions under lantern globes, the tops of which were sealed with a thin layer of cotton between squares of cheese cloth. The spores from each uredinium were transferred to seedlings in a separate pot so that 6 single-pustule cultures were thus established. Bulk transfers also were made at the same time to seedlings kept in the muslin cages. The variety Harvest Queen rather than Kanred was used as the host in these studies because it is susceptible to more physiologic forms of leaf rust than Kanred. For comparison, transfers of physiologic form 9 were made at the same time and in identically the same way as those of culture No. 199. All cultures were watched carefully during the incubation period and the dates for flecking and eruption of the uredinia, as well as other characters, were noted. The results of this experiment are given in table 1. It is clearly shown that not only is the

TABLE 1.—*Comparison of the incubation and developmental periods and size and color of uredinia of culture No. 199 and physiologic form 9 of Puccinia triticina on Harvest Queen wheat in the greenhouse. Inoculated April 15, 1928*

Culture or form No.	Bulk or single pustule	Number of days from inoculation to			Size of uredinia	Color of uredinia
		Flecking	Sporulation	Full development		
199	bulk	10	14	23	small	light orange
199–1	s. p.	11	14	23	"	" "
199–2	s. p.	10	13	22	"	" "
199–3	s. p.	10	15	23	"	" "
199–4	s. p.	10	15	23	"	" "
199–5	s. p.	10	15	23	"	" "
199–6	s. p.	10	14	23	"	" "
p.f. 9	bulk	5	8	13	large	orange red
9–1	s. p.	5	8	13	"	" "
9–2	s. p.	5	8	13	"	" "
9–3	s. p.	5	8	13	"	" "
9–4	s. p.	5	8	13	"	" "
9–5	s. p.	4	7	13	"	" "
9–6	s. p.	5	8	13	"	" "

incubation period longer for culture No. 199 than for physiologic form 9, but also that the time required for the culture to reach its full development is longer. Furthermore, it became evident that the spores of culture No. 199 were much lighter in color than those of physiologic form 9 and the uredinia were smaller in size. The latter point might suggest to some investigators that these peculiarities were due to the resistance of the host

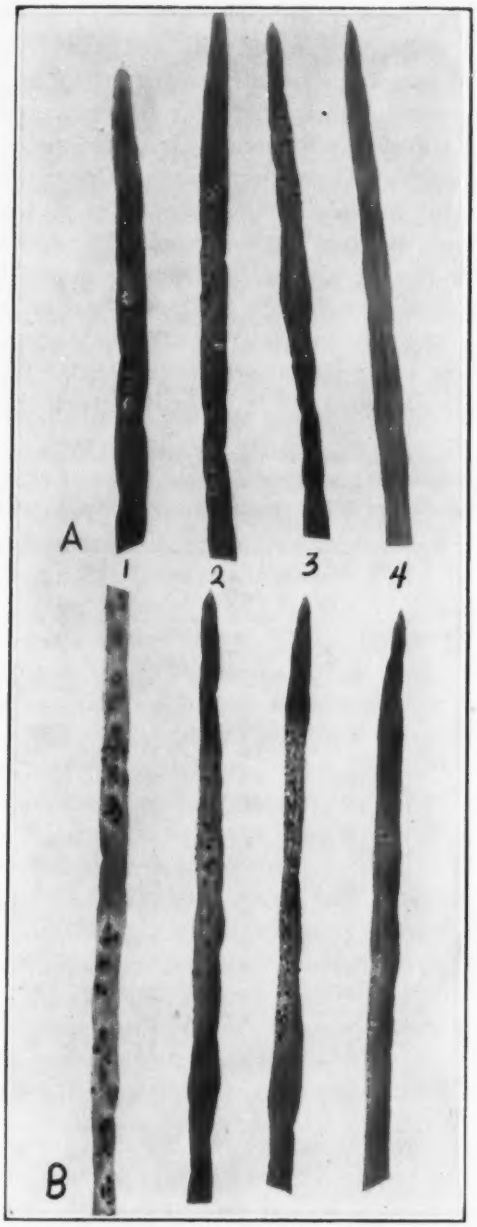

FIG. 1. Leaves of Harvest Queen wheat collected March 28, 1929, inoculated with (A) culture 199, and (B) physiologic form 9 of *Puccinia triticina*. 1. Inoculated March 2, 1929. 2. Inoculated March 5, 1929. 3. Inoculated March 14, 1929. 4. Inoculated March 20, 1929.

to that culture of leaf rust. It is true that in one form of resistance there is a reduction in the size of the uredinia. Small uredinia due to this type of resistance, however, are invariably accompanied by necrosis around the uredinia, and there is very little extension of the incubation period. Small or even minute uredinia on highly resistant varieties develop nearly as quickly as large normal uredinia on susceptible varieties. In the case of culture No. 199, however, there was no necrosis of the tissues surrounding the uredinia and the length of the incubation and developmental stages was strikingly longer than for physiologic form 9.

The difference in the length of incubation period is clearly illustrated in figure 1, showing eight leaves of Harvest Queen wheat, the lower four inoculated with physiologic form 9, and the upper four with culture 199. Each vertical pair of leaves was inoculated on the same date progressively later from left to right. Pair No. 1 was inoculated on March 2, No. 2 on March 5, No. 3 on March 14, and No. 4 on March 20, 1929. All leaves were collected on March 28. In Pair No. 4 eight days after inoculation the leaf inoculated with physiologic form 9 showed uredinia just breaking through the epidermis, while no uredinia had developed on the leaf inoculated with culture 199. Pair No. 3 showed physiologic form 9 well developed at the end of 14 days, while culture 199 was just rupturing the epidermis, only a few of the uredinia being completely uncovered.

Studies on the comparative length of the incubation period of culture 199 and physiologic form 9 were continued in the greenhouse during the fall and winter of 1928–1929. Nine different transfers of both cultures

TABLE 2.—*Comparison of the incubation periods of physiologic form 9 of Puccinia triticina and culture No. 199 on Harvest Queen wheat in the greenhouse, 1928–1929*

Date of Inoculation	Physiologic form 9		Culture 199	
	Number of days from inoculation to			
	Flecking	Sporulation	Flecking	Sporulation
Oct. 23	5	8	13	19
Nov. 12	6	8	11	14
Feb. 5	6	8	10	14
Mar. 2	4	7	8	12
Mar. 5	5	8	9	13
Mar. 9	4	7	9	12
Mar. 20	4	7	6	12
Mar. 26	4	7	5	12
Mar. 29	4	8	7	14
Average	4.6	7.5	8.6	13.5

were made to the variety Harvest Queen at different times during the season and the dates on which flecking and sporulation was first observed were recorded. These data are summarized in table 2. It is very evident that the average interval from inoculation to the beginning of sporulation was nearly twice as long for culture 199 as for physiologic form 9.

A set of the varieties having differential reaction to leaf rust was inoculated with culture 199 on April 20, 1929. These are the varieties used in the identification of physiologic forms and their reaction could be expected to shed some light on the question of whether the long incubation period of culture 199 was due to resistance of the host variety. A similar set was simultaneously inoculated with physiologic form 9. Notes were secured on the length of the incubation and developmental periods in each case (Table 3). It will be noted that the incubation period of form 9 was only

TABLE 3.—*Comparison of the incubation and developmental periods of two cultures of P. triticina on varieties of wheat having differential reaction in the greenhouse. Inoculated April 20, 1929*

Variety	C. I. No.	Culture or form No.	Number of days from inoculation to			Size of uredinia	Color of uredinia
			Flecking	Sporulation	Full development		
Malakof	4898	199	8	12	22	small	light orange
		p.f. 9	5	7	12	large	orange red
Mediterranean	3332	199	8	13	23	small	light orange
		p.f. 9	5	8	13	minute	orange red
Democrat	3384	199	8	13	23	small	light orange
		p.f. 9	5	8	13	minute	orange red
Hussar	4843	199	9	13	24	small	light orange
		p.f. 9	5	7	12	"	orange red
Webster	3780	199	8	12	22	small	light orange
		p.f. 9	5	7	11	large	orange red
Norka	4377	199	8	12	22	small	light orange
		p.f. 9	5	8	11	large	orange red
	3756	199	9	13	23	small	light orange
		p.f. 9	5	8	13	"	orange red
	3778	199	9	13	23	"	light orange
		p.f. 9	5	8	13	"	orange red
	3779	199	8	13	23	"	light orange
		p.f. 9	5	7	11	large	orange red
	3747	199	8	12	22	small	light orange
		p.f. 9	5	7	12	large	orange red
Kanred	5146	199	8	13	23	small	light orange
		p.f. 9	5	7	12	large	orange red

slightly longer on the four resistant varieties, Mediterranean, C. I. 3332, Democrat, C. I. 3384, C. I. 3756, and C. I. 3778, than on the other selections. The incubation period and time required to reach full development on all varieties were much longer for culture 199 than for physiologic form 9.

TABLE 4.—*Comparison of the length of incubation periods of several cultures of Puccinia triticina in the greenhouse, Manhattan, Kansas, 1929*

Variety	C. I. No.	Culture No. 369–6		Culture No. 308		Culture No. 367		Culture No. 199	
		Number of days from inoculation to							
		Flecking	Sporulation	Flecking	Sporulation	Flecking	Sporulation	Flecking	Sporulation
Malakof	4898	5	8	5	8	5	...	9	14
Mediterranean	3332	5	9	5	9	5	8	8	16
Democrat	3384	5	9	5	9	5	8	8	16
Hussar	4843	5	8	5	9	5	9	9	16
Webster	3780	5	8	5	...	5	...	9	16
Norka	4377	5	8	5	8	5	...	9	16
	3756	5	8	5	...	5	9	9	14
	3778	5	8	5	8	5	8	8	16
	3779	5	8	5	9	5	8	8	14
	3747	5	9	5	...	5	...	9	16
Kanred	5146	5	8	5	8	5	8	8	14
		—	—	—	—	—	—	—	—
Average		5	8.2	5	8.5	5	8.2	8.5	15.2

It is true that some of the varieties showed some necrosis and other signs of resistance to culture 199, but that did not seem materially to affect the length of the incubation period. This experiment was also repeated in the greenhouse in 1929 (Table 4). Here again it will be noted that the average interval from inoculation to sporulation for culture 199 was nearly twice that of the three other cultures.

Another experiment, conducted in 1929, affords further evidence of a longer incubation period for this culture. On March 30 a group of 38 varieties and selections were inoculated with a culture of physiologic form 3 and a similar group with culture 199. The varieties comprised 31 selections all highly resistant to physiologic form 9, 4 selections susceptible to that form and the Australian varieties, Clarendon, Warren, and Thew. Form 3 produced heavy infection on 30 of the selections, a type-2 infection on five selections, and none on three. Culture 199 seemed less virulent, but produced a type-3 infection on 20 selections, a type-2 on eight, a 0 or 0–1-type on five, and an x-type on two. All plants of two other selections died and were discarded. It readily can be seen that, although culture 199 was not quite so virulent as physiologic form 3, the average reaction of the group of varieties to the two cultures was about the same, there being approximately the same number of varieties susceptible and resistant to each culture of rust. The notes on the incubation periods showed wide difference in the time required for the production of visible evidence of flecking and sporulation (Table 5).

TABLE 5.—*Comparison of the average incubation periods of culture 199 and physiologic form 3 on seedlings of 38 varieties of wheat in the greenhouse, 1929*

Total number of selections	Physiologic form 3		Culture 199	
	Number of days from inoculation to			
	Flecking	Sporulation	Flecking	Sporulation
38	5	8	7.4	13.0

In this case flecking was noted for physiologic form 3, an average of nearly two and a half days earlier than for culture 199, while the beginning of sporulation was 5 days earlier.

It already has been stated that all other cultures of *Puccinia triticina* which have been tested in the greenhouse at Manhattan have been characterized by about equally long incubation periods. More than 200 cultures have been studied in detail during the period, 1926 to 1929, inclusive.

Many proved to belong to physiologic forms already described, while others seemed to be new or undescribed forms. Among all these cultures the only one with a strikingly long incubation period was culture 199. All others had developmental periods very similar to that of physiologic form 9. They also all had the same orange red color and large uredinia on susceptible varieties. Culture 199 was not compared with all cultures under study, but was compared with several cultures selected at random both in 1928 and 1929. Inoculations were made with several cultures at the same time and notes taken on date of flecking and sporulation. Data from one such experiment have been presented in table 4. The results of similar experiments conducted in 1928 and 1929 are presented in table 6. It is

TABLE 6.—*Comparison of incubation period and color of uredinia of several cultures of Puccinia triticina on Harvest Queen wheat in the greenhouse 1928 and 1929*

Culture No.	Physiologic form	Number of days from inoculation to			Color of uredinia
		Flecking	Sporulation	Full development	
1928					
199		10	14	20	light orange
119–3	5	5	8	12	orange red
306	9	5	8	12	" "
307	9	5	8	12	" "
198–1		6	8	12	" "
154–4–1	5	6	8	12	" "
170–3		5	8	12	" "
1929					
199		7	14	19	light orange
stock	9	4	7	10	orange red
121–2–1–3	3	4	7	10	" "
119–3–5	5	4	7	10	" "
389–4		4	7	10	" "
157–2–A		4	7	10	" "
362–4		4	7	10	" "

apparent that culture 199 is distinctive from all others, both in length of the incubation period and in spore color as reflected in color of the uredinia.

The differences noted above were sufficiently striking to raise the question whether also there might not be differences in spore dimensions. A number of mounts of spores of form 9 and culture 199 therefore were made and studied. In general characteristics the spores of culture 199 seemed very similar to those of form 9. Their walls were seemingly slightly thinner and therefore somewhat more irregular in shape than those of the spores of

form 9. The lighter color of the spores was not so evident under the microscope as in the spore masses of the uredinia. In spore dimensions there seemed to be a slight difference between the two cultures, although the number of measurements was not large enough to give positive results. Fifty spores each of culture 199 and form 9 were measured for length and width. Although spores of leaf rust are nearly globular they usually are slightly larger in one dimension than another, or have irregularities that affect the spore shape. The results of these measurements gave the following average dimensions in microns:

	Length	Width
Form 9	26.87 ± .159	25.02 ± .134
Culture 199	23.48 ± .140	22.71 ± .125
Differences	3.39 ± .211	2.31 ± .183

These figures seem to indicate that the spores of culture 199 are definitely smaller than those of physiologic form 9, and that the differences are statistically significant. In both length and width measurements the probable error of the differences is less than ten per cent of the difference. The number of measurements is too small, however, to give entirely reliable results.

To determine whether culture 199 differs from known physiologic forms in its behavior on varieties of wheat having differential reactions, or is similar to some known form, several experiments were conducted in 1928 and 1929. It was found extremely difficult, however, to obtain accurate rust readings on differential varieties because of the extremely long developmental period. For all other cultures of rust under investigation readings could be made 12 to 14 days after inoculations. Readings could seldom be made on culture 199 until the 18th day and frequently not until 20 days after inoculation. Seedlings of differential varieties of wheat, inoculated when 10 days old, were 30 days old by that time. The prophylla of seedlings of this age usually have turned yellow and frequently are extremely etiolated. As a result, the plants usually had passed the stage where satisfactory rust readings could be made before culture 199 was fully developed. Very little can be determined about necrosis and flecking due to rust on chlorotic leaves. Many sets of differentials were in such poor condition when the rust had finally developed as to make accurate readings impossible and they were therefore discarded. In a few cases, however, rust readings were made. For reasons given, the results were somewhat variable.

In fact, no completely satisfactory readings have yet been secured on this culture. The most satisfactory readings give reactions somewhat similar to those for physiologic form 10. The comparative infection-type readings for that form and for culture 199 are as follows:

Differential variety	C. I. No.	Infection type	
		Physiologic form 10	Culture No. 199
Malakof	4898	4	3–4
Norka	4377	4	3–4
Mediterranean	3332	1–2	2– to 2
Democrat	3384	1–2	2–3
Hussar	4843	1–2	2–3
Webster	3780	4	3–4
(Unnamed)	3747	3–4	3
"	3756	4	2
"	3778	4	1–2
"	3779	4	3

The differential reaction of the two rusts on Democrat, Hussar, C. I. 3756, and C. I. 3778 makes it seem certain that culture 199 is not physiologic form 10, and, as it resembles no other described form, it probably is a new form.

It should be stated here that no straight type-4 infection has been noted for culture 199. As shown above, a variety occasionally exhibited a susceptibility classified as 3–4, but the copious production of large normal uredinia characteristic of type 4 was not seen. The uredinia always were smaller and less abundant for culture 199. The small size and light color of the uredinia probably had considerable effect on the infection types assigned a variety in making rust readings. Thus it appears that culture 199 is a different physiologic form, although it somewhat resembles physiologic form 10. Aside from the differential reaction noted above, the long incubation period, light-color spores, and small uredinia of culture 199, also, would place it in a separate category.

CONCLUSIONS

When all characteristics of culture 199 are considered, the possibility of its origin through mutation is strongly suggested. There is, however, no direct evidence in proof of the point. Even circumstantial evidence is entirely too meager to justify such a conclusion. The mutations noted by Newton and Johnson[8] occurred under observation and therefore cannot be

[8] *Loc. cit.*

questioned. The origin of culture 199, on the other hand, is entirely unknown.

It is certain, however, that culture 199 is an aberrant type of *P. triticina*. It differs distinctly from all other cultures yet studied in the greenhouse at Manhattan, Kansas, in length of incubation period, spore color, and size of uredinia on susceptible varieties. Those characters also were evident in the culture when collected in the field. It was noticed and collected because it appeared very late in the season on a highly resistant variety and was characterized by a small number of minute, pale uredinia. Regardless of the origin the culture is interesting in the constancy with which the variations noted above were expressed in repeated transfers. The writer has frequently noted a sparse infection of leaf rust on highly resistant varieties in the field late in the season. Such infections usually were characterized by minute uredinia, frequently of very light color. These usually were regarded as late-developing infections due to the resistance of the host. Such actually may have been the case, but, on the other hand, they may have been due to the presence of an aberrant type of leaf rust similar to that of culture 199.

SUMMARY

An aberrant form of leaf rust of wheat, *Puccinia triticina*, was found on a highly resistant strain of Mediterranean wheat, Texas 3015–63, at Denton, Texas, in the spring of 1927. This culture is discussed as culture 199.

Greenhouse experiments have shown that this form differs from all other cultures studied at Manhattan, Kansas, in length of incubation period, color of spores, size of uredinia, and spore dimensions.

The period from inoculation to flecking averages about three days longer, and that from inoculation to sporulation about seven days longer, than for other known forms. The time required to reach full development also is much longer for culture 199.

The spores are a lighter orange in color than those of other forms and the uredinia are smaller. Spore measurements indicated that spores of culture 199 were slightly smaller than those of form 9.

It was proved that the peculiar behavior of culture 199 was not due to the resistance of the host variety.

On differential varieties the culture gave reactions indicating that it was an undescribed physiologic form. The aberrant nature of the culture, however, makes its identity doubtful.

The aberrant type may have arisen through mutation, but only meager evidence is available on that point.

THE RELATION OF TYPE OF TOPPING TO STORAGE LOSSES IN SUGAR BEETS[1]

C. M. Tompkins and S. B. Nuckols[2]

INTRODUCTION

In an examination of stored beets at Lewiston, Utah, on December 20, 1927, after they had passed through the washer in the factory mill, it was noted that storage rots were doing considerable damage. Fully 50 per cent of the beets in the hopper showed more or less decay, the amount varying from small involvements of crown to total decay of the root. The commonest type of rotting in this stored material was what is called in this paper "crown rot" to distinguish it from the other decays of beets that arise from wounds or bruises or that may arise from the drying of the small side roots or the tapering tip of the main root. This distinction is made, although probably the same decay-producing organisms are involved in the rotting, because of the clear-cut relation of the crown-rot type to the invasion of the crown surface of the beet exposed by topping. In the observations made at Lewiston, Utah, and which were later repeated at Logan, Utah, beets with the greater portion of the crown removed by topping showed the most rotting. Those beets on which a large portion of the crown remained were almost free from decay, while beets topped at the line of the lowest leaf scar (the approved commercial method of topping) showed approximately 50 per cent in various stages of decay. Those beets topped one-fourth inch or more below the base of the lowest leaf scar were in nearly all instances severely attacked by decay organisms.

These observations led the writers to begin investigations upon the nature of crown rot of stored beets, its seriousness, and the connection of the rot to the type of topping practiced. The investigations undertaken have involved some isolation work along with inoculations to indicate the organisms concerned with decay of sugar beets in storage piles, and a thorough examination of the beets in the storage pile to determine what relations existed between crown rot and the type of topping which had been given the individual beets.

[1] The writers are indebted to Dr. B. L. Richards, Utah Agricultural Experiment Station, and Dr. G. H. Coons, Office of Sugar Plants, U. S. Department of Agriculture, for kindly suggestions and critical reading of the manuscript. Appreciation is also expressed to The Amalgamated Sugar Company, Ogden, Utah, in whose laboratories all chemical analyses were made.

[2] Assistant Pathologist and Associate Agronomist, respectively, Office of Sugar Plants, U. S. Department of Agriculture.

THE ORGANISMS CAUSING CROWN ROT

From among 10,945 beets examined at Logan, Utah, 400 sugar beets showing definite symptoms of the crown rot described were selected for isolation purposes. Two small fragments from margins of lesions were cut from each beet under aseptic conditions and planted on potato-dextrose agar to which had been added several drops of 10 per cent lactic acid to prevent bacterial contamination. Strong fungous growth was obtained from nearly all within 36 hours. Transfers were made to tubes of potato-dextrose agar by picking off hyphal tips at the edge of the colonies by means of a sterile platinum needle. The cultures in the tubes were incubated for one week to insure sporulation, after which cultures were made from single spores by plating. From the 800 tissue plantings, 381 cultures of *Phoma betae* and 339 isolations of two species of Fusarium (*Fusarium culmorum* and *Fusarium* sp., similar to *F. oxysporum*), were obtained, the remaining 80 tissue plantings failing to give fungous growth.

This large number of tissue plantings was made in order to determine the relative frequency of *Phoma betae* and the two species of Fusarium in diseased tissue. This was suggested as a result of isolations made from a limited amount of material collected at Lewiston, Utah, as the beets were dropping into the hoppers in the factory immediately following washing. Isolations from the Lewiston beets yielded *P. betae* mainly, but two different species of Fusarium were also frequently obtained. The results of isolations made from rotted sugar beets collected at Lewiston and Logan, Utah, indicate that *P. betae* is extremely important as a septic factor in sugar beets, since it was associated in more than one-half of the cases of crown rot from which isolations were made. Two species of Fusarium are also concerned in this type of decay. These have been classified as follows by Dr. C. D. Sherbakoff.[3]

"Isolations 258 and 263: *Fusarium culmorum;* the strain with commonly shorter, 3-septate conidia as in *F. discolor* var. *triseptatum* Sherb. The last named fungus appears to be identical with this strain of *F. culmorum.*" (Fig. 1, *1* and *2*.)

"Isolations 262 and 264: *Fusarium* sp.; in microconidia, is similar to *F. oxysporum;* macroconidia few and poorly developed or none (hence the difficulty of identification); on agars with glucose, color often purplish slate; chlamydospores and other characters of section Elegans." (Fig. 1, *3* and *4*.)

[3] The writers wish to express their appreciation to Dr. C. D. Sherbakoff, Plant Pathologist, Tennessee Agricultural Experiment Station, for study given the various isolations of Fusarium sent him and for his suggestions as to the names provisionally to be assigned to these organisms.

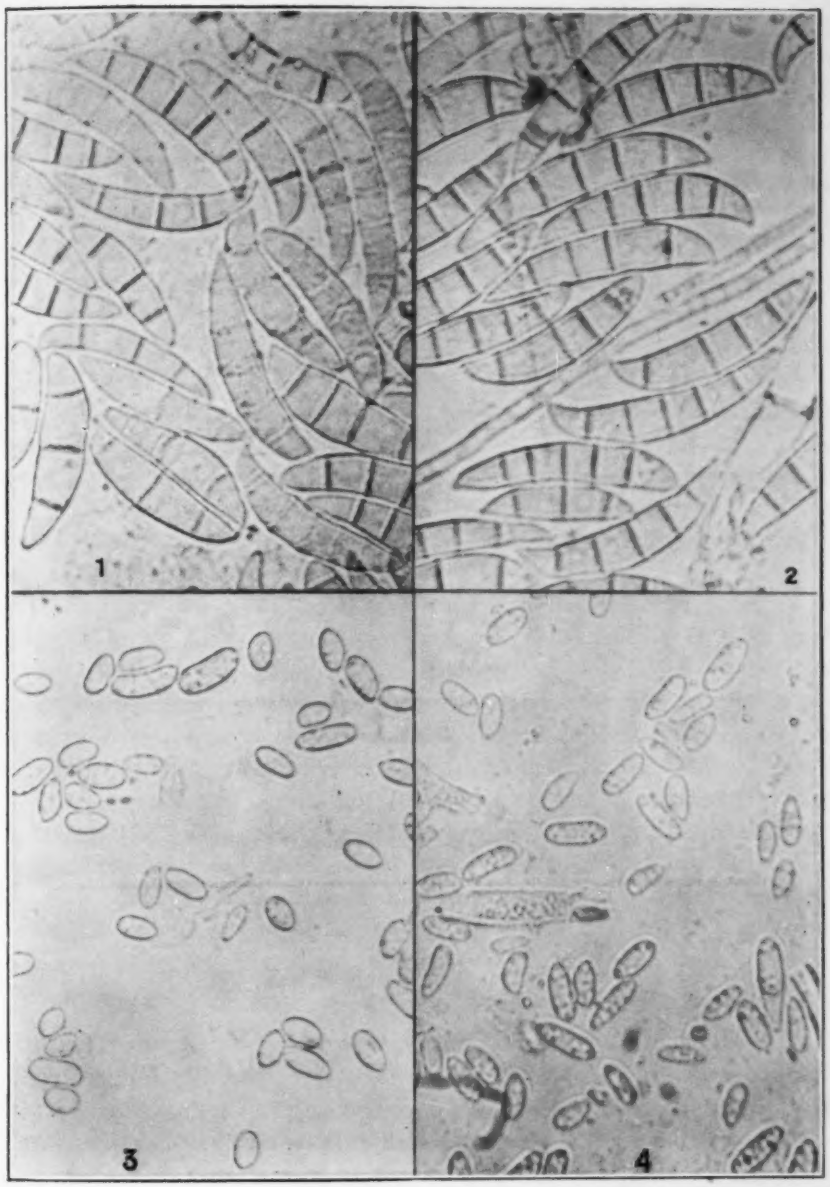

FIG. 1. *1* Macrospores from *Fusarium culmorum*, Isolation 258; *2* Macrospores from *F. culmorum*, Isolation 263; *3* Microspores from *Fusarium* sp. from Isolation 262; *4* Microspores from *Fusarium* sp., Isolation 264 (×1000). Photomicrographs through courtesy of Dr. C. D. Sherbakoff.

TABLE 1.—*Inoculations of healthy roots of sugar beets with Phoma betae and Fusarium* sp. (*Inoculated February 15, 1928; read March 31, 1928*)

Organism	Isolation No.	Source of organism	No. of beets inoculated	No. of beets diseased in 45 days	Percentage diseased in 45 days	Controls	
						No. of beets used	No. of beets healthy at close of 45-day period
Phoma betae	259	Logan, Utah	70	56	80.0	10	10
" "	260	" "	70	60	85.7	10	10
" "	261	" "	70	70	100.0	10	10
" "	265	Lewiston, "	70	70	100.0	10	10
Fusarium culmorum	258 and 263	Logan, "	70	57	81.4	10	10
		Lewiston, "	70	55	78.5	10	10
Fusarium sp. (Similar to *Fusarium oxysporum*)	262 and 264	Lewiston, "	70	40	57.1	10	10
		" "	70	16	22.8	10	10

For inoculation purposes a large number of healthy sugar beets were treated ten minutes in a solution of 1 to 1000 mercuric chloride solution and then washed successively in tap, distilled, and sterile water. With a large, stainless steel knife, previously treated by dipping in 95 per cent alcohol and flaming, a thin slice of tissue was cut from each beet parallel to the original topped area, thus exposing fresh tissue free from cork cells. Week-old cultures of *Phoma betae* (represented by four isolations) and of the two species of Fusarium (represented by two isolations of each species) on prune agar were used for the inoculations. The inoculum, consisting of a block of agar approximately 2 cm. square by 0.5 cm. deep, plus growth, was placed on the freshly exposed cut surface, with the fungus in direct contact with the beet tissue. A piece of sterile cotton moistened with sterile water was then placed over the inoculum and held in position by means of a strip of adhesive tape. The beets, after inoculation, were placed in glassine bags and stored in a cellar where the air temperature was approximately 4° to 6° C. Results of inoculations are given in table 1. Reisolations were made in each case from beets in which decay had been induced artificially and the fungus again obtained in pure culture.

From this table it is clear that rotting of stored sugar beets can be inaugurated in a high percentage of cases by *P. betae* and the two Fusarium species tested, if these fungi are introduced in considerable mass upon freshly wounded crown tissue. It can not be said that the variation in percentages of disease production is indicative of the aggressiveness of the particular pathogenes, since variations in the reaction of the host, in storage conditions, and other factors may have influenced the results. We may, however, from these inoculations, definitely assign to certain pathogenes a causal connection with the decay of sugar beets occurring in storage piles. Probably other fungi are occasionally concerned in this decay, and many species of fungi and bacteria invade the attacked tissues as secondary invaders.

THE RELATION OF CROWN ROT TO THE TYPE OF TOPPING

Sugar beets which had been commercially topped, stored at Logan, Utah, in dirt pits, and covered with a thin layer of moist sand were available for rather complete study. These beets were removed from the pits after being in storage 84 days. The conditions for storage were presumably as good as, if not better than, would be found in commercial storage. It is not assumed that the same amount of rot would be found in all commercial storage piles, since the period was greater than the ordinary period of storage. It is probable, however, that the disease conditions prevalent were approximately the same as with similar beets in commercial storage piles where storage rots were serious.

In examining these beets considerable variation was found in the amount of crown taken off in the topping process. The reason for this is apparent when the methods used in the field are considered. In handling the beets for topping with a hooked beet knife, the laborer lifts the beet from the ground to his left hand by use of the hook. One quick stroke usually severs the crown from the root at approximately a right angle to the longitudinal axis of the beet. The instructions are to make this cut at the base of the lowest leaf scar, but unavoidable variations enter. The laborer usually does not attempt to correct imperfections in topping, with the result that beets are delivered to the factory with the majority approximately correctly topped, while the others are topped above or below the lowest leaf scar.

In order to make a careful study of stored beets and the relation of topping to the amount of fungous decay, five arbitrary divisions of the types of topping were made. The beets in the storage pit used for this investigation were examined and placed in the class which most nearly represented its individual type of topping. In cases where the cut was slanting instead of at a right angle to the longitudinal axis, the beets were assigned to the class represented by the lowest angle of the cut.

For convenience, these classes are designated:

Class a—To include all beets topped one-half inch or more above the lowest leaf scar, leaving a large amount of crown attached to the root.

Class b—To include all beets topped approximately one-fourth inch above the lowest leaf scar, leaving a smaller amount of crown attached to the root.

Class c—To include all beets topped on a line at the base of the lowest leaf scar. This would remove all of the leaf scars from the beet, leaving all of the root tissues intact with no crown adhering.

Class d—To include all beets topped one-fourth inch below the lowest leaf scar. This would remove approximately one-fourth inch of the root with the crown.

Class e—To include all beets topped one-half inch below the lowest leaf scar. This would remove one-half inch or more of the root with the crown. (Fig. 2, A.)

Class *c* represents the standard commercial topping. Commercial companies do not object to beets topped as represented by Classes *c*, *d*, and *e*, although they recognize as wasteful the topping described in Classes *d* and *e*. The companies do object to topping as represented by Classes *a* and *b*, and, through tare sample determinations, they arrive at the extra crown weight of such beets and deduct the same from the gross weight of beets delivered.

In this study all beets were classified as diseased or healthy according to the presence or absence of rotted areas on the cut surface. Beets showing a healthy condition of this cut surface, but having decayed areas in

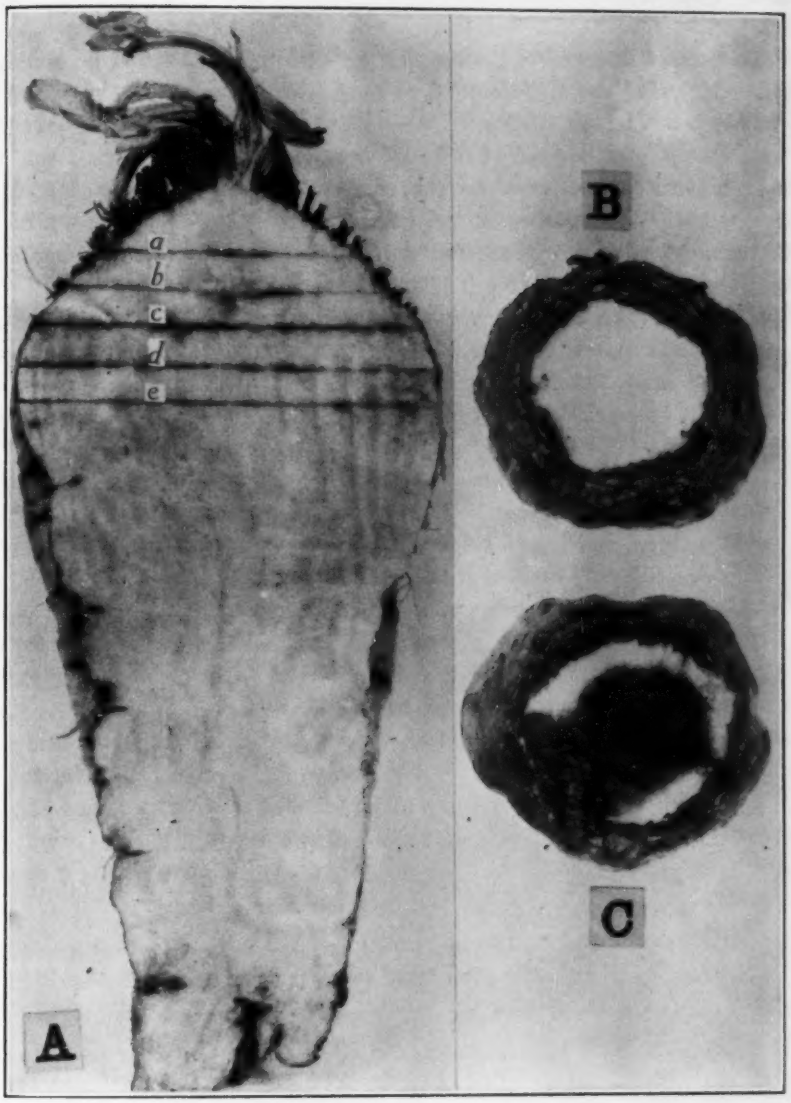

FIG. 2. A, Chart adopted for classification of sugar beets according to type of topping. Line *c* marks the point of the lowest leaf scar which is the point of commercial topping. B, Healthy and C, diseased crowns of sugar beets topped at *a*. Storage period 84 days.

other portions of the root arising from other injuries, were classed as healthy because their decay bore no relation to injury induced by removal of the crown.

The beets were removed from this pit and were classified according to the five types of topping detailed above. The presence or absence of decayed areas on the cut surface of the root was then noted. Beets free from decay in the crown portion of the root were classed as healthy. Beets with one or more decayed areas on the cut surface were classed as diseased. There was considerable variation in the amount of decay on individual beets, some having very small decayed areas and others showing badly rotted areas.

The Class *c* type of topping is the approved commercial method of topping, and the greatest number of beets examined were found to be topped at the line *c* (Fig. 2, A). More than 50 per cent of the beets were properly topped and there seemed more tendency to leave a part of the crown on the beet than to top the beet too low. The results of this classification of the beets and the relative amount of crown rot in the various classes are shown in table 2. The definite and consistent trend in percentages of rotted and sound beets in the various established classes indicates that the type of topping has a direct effect upon the incidence of infection by parasitic fungi in stored beets. The beets in Class *c*, conforming to the approved commercial type of topping, were found to be 37 per cent healthy and 63 per cent diseased at the end of the 84-day storage period. Lower topping increased the amount of decay so that approximately four-fifths of the beets of Classes *d* and *e* showed various degrees of crown rot. Approximately two-thirds of the beets topped at the line *b* were healthy, while in those topped at the line *a* there was approximately only one diseased beet in seven. These strongly contrasting figures indicate close relation of type of topping to the numbers of individuals showing crown rot in storage, and that there is a marked increase in rotting as the plane of topping is lowered.

TABLE 2.—*Relation of type of topping to the keeping qualities of stored sugar beets: Commercial beets from Logan, Utah, stored in covered pits for 84 days*

Type of topping	Total		Number of beets		Percentage	
	No. beets	Percentage	Healthy	Diseased	Healthy	Diseased
a	730	6.67	632	98	86.58	13.42
b	2933	26.80	1999	934	68.16	31.84
c	5616	51.31	2085	3531	37.13	62.87
d	1086	9.92	198	888	18.23	81.77
e	580	5.30	118	462	20.34	79.66
Totals ..	10945	100.00	5032	5913	45.98	54.02

There also appeared to be marked variation in the degree of involvement by crown rot in the various classes of beets. Table 3 gives the results of determinations made upon 90 beets representative of the rotted beets of the five classes. The actual amounts of sound and diseased tissue were determined for these samples and the percentage of involvement computed. These percentages are believed to be fair indexes of the conditions of the rotted beets in the various classes (Figs. 2 and 3). On this basis, as can be seen from the table, the rotted beets of Class *c* had approximately 14 per cent of their tissue affected at the end of the storage period. The tissue involvement increased with lower topping, rotted beets from Classes *d* and *e* showing, respectively, 4 and 8 per cent more of their tissue decayed than was found in Class *c* beets. On the other hand, Classes *a* and *b* beets

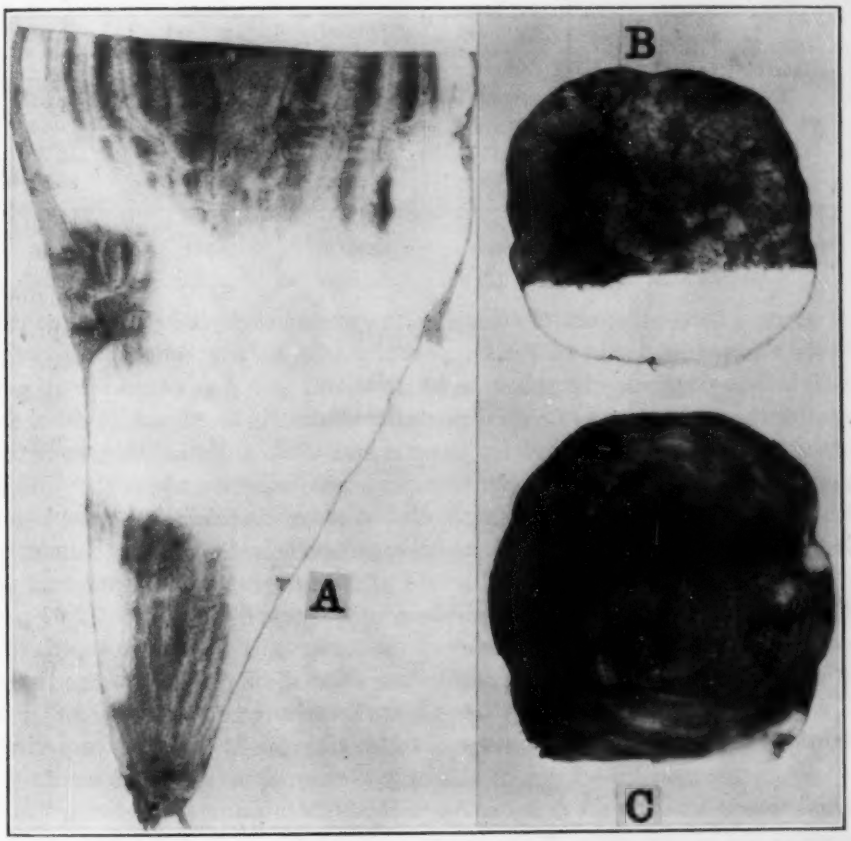

FIG. 3. A, Longitudinal section of sugar beet topped at *d*, showing advanced stage of decay. B, Healthy, C, diseased crowns of sugar beets topped at *e*. Storage period 84 days.

showed, respectively, 8 and 6 per cent *less* tissue involvement than Class *c* beets.

The percentages of involvement in the rotted beets, shown in table 3, when considered in connection with the actual numbers of sound and rotted beets of the respective classes, as shown in table 2, reveal a very significant situation as to total weight of beet tissue in the various classes affected by rot.

TABLE 3.—*Relation of type of topping to the extent of diseased tissue in stored sugar beets. Storage period 84 days. The beets used in this test showed distinctly that the origin of infection was from the crown scar*

Type of topping	Number of beets examined	Gross weight of beets (grams)	Weight of healthy tissue (grams)	Weight of diseased tissue (grams)	Percentage of total tissue diseased
a	8	6124	5835	289	4.72
b	16	13695	12553	1142	8.35
c	21	18976	16189	2787	14.69
d	18	14116	11816	2300	16.29
e	27	17669	13626	4043	22.88
Totals	90	70580	60019	10561	Average 14.96

Class *c* beets came out of storage with approximately 63 per cent of the beets affected with crown rot. Approximately 15 per cent of the total weight was decayed. In this case, there would be a loss of nearly 10 per cent of the total weight of *c* beets due to crown rot in storage. The shrinkage due to rot is still greater for Class *d* and Class *e* beets, being approximately 14 and 17 per cent of the respective total weights, which shows that topping at the line *d* or *e* has grave disadvantages in addition to the regular topping loss which comes in the field. Class *b* beets had approximately 32 per cent of the individuals diseased and an average of 8.3 per cent of the tissue damaged. This is equivalent to shrinkage from rot of 2.66 per cent of the total weight of all beets of this class. In Class *a* 13.4 per cent of the individuals were diseased, and these showed 4.7 per cent of the tissue decayed. This indicates that Class *a* beets sustained a loss of only 0.63 per cent of their total weight from crown rot during the 84-day storage period.

From common knowledge of the nutritional requirements of the fungi and bacteria which cause decay of beets, it is safe to assume that in the processes of decay the sugar of the involved tissue would be destroyed by the attacking organisms. The accuracy of this assumption is borne out by the analyses given in table 4 in which composite samples of many beets, but with varying percentage of rotted tissue, were used.

The samples were made up of beets showing approximately the same ratio of diseased and healthy tissue as was found in the general run of beets in the different classes from the storage pit. It is noticeable that Class *a* beets, which, because of their high tops, probably had the lowest amount of total sugar, came through the storage period with the highest sugar content and highest purity. It will be noted from the table that the sugar percentage of the beets dropped in close proportion to the percentage of rot and that the purities were strongly affected. The results shown in this table give some indication of how important losses of this type may become.

TABLE 4.—*Post-storage analyses of differently topped sugar beets. Storage period 84 days*

Type of topping	Healthy tissue in sample	Diseased tissue in sample	Sugar in beets	Purity of juice
	Per cent	Per cent	Per cent	Per cent
a	99.35	0.65	18.09	84.3
b	97.33	2.67	17.96	82.7
c	90.77	9.23	17.13	80.6
d	86.92	13.08	15.37	73.8
e	81.77	18.23	14.51	70.1

It is obvious from the data presented that from the standpoint of crown-rot prevention, the practice of leaving a higher crown in topping must be considered. Offsetting the savings which come from prevention of loss of extractible sugar by decay, there must be taken into account the general effects on sugar and purity which arise from the well-known lower sugar and high mineral content of the sugar-beet crown. Factory operators object to any practice which tends to lower sugar content or purity of juice in the sliced product because these items tend to increase manufacturing difficulties and cost of production.

In the beets studied in these experiments there were no indications of sprouting of the stored beets. The present paper offers no data on the effect of greater crown portions on the sprouting of beets stored under conditions favorable to the growth of sprouts on the beets. In case sprouting were brought about by storage conditions, there would presumably be the greatest growth of sprouts on those beets having the greatest amount of crown, and this also would offset to some degree the savings indicated by the crown-rot prevention. In average seasons, however, air temperatures during the storage period are generally low enough to preclude the growth of sprouts.

Since work on this problem did not commence until the end of the factory campaigns in the Western States, no harvest data are available on the percentage of sugar, purity, and ash analyses of beets corresponding to the classes set up in this paper. As a substitute for freshly harvested beets, some analyses were made of beets kept in cold storage 84 days. These beets had been held at a temperature which precluded infection and were in a crisp condition and free from all signs of decay. When harvested the entire crown was left on the beet root. Although beets undergo some chemical changes in storage, it is believed that the relations in the various portions in these beets approximate those of freshly harvested beets. These whole beets were sectioned so as to obtain material representing the portion of the beet which would be removed by successive topping at the five arbitrary points *a*, *b*, *c*, *d*, and *e*. The portion removed by topping at the line *a* contained relatively the lowest sugar content and the highest ash content. The portions removed by lower topping had a successive increase in sugar content and a successive decrease in ash content (Table 5). These data correspond closely with data published by other authors who worked with fresh beets.

TABLE 5.—*Post-storage analyses of sections of the crown and root of healthy sugar beets. Analyses of 38 beets kept in cold storage 84 days. Percentage of ash based upon weight of dry substance*

Section of beet	Sugar	Soluble ash	Insoluble ash	Total ash
	Per cent	Per cent	Per cent	Per cent
Above *a*	8.4	5.60	10.51	16.11
Between *a* and *b*	11.6	4.17	5.92	10.09
Between *b* and *c*	14.9	2.67	2.74	5.41
Between *c* and *d*	15.1	3.03	1.58	4.61
Between *d* and *e*	16.0	2.64	1.50	4.14
Below *e*	16.7	2.55	1.45	4.00

By use of the data obtained from analyses of various sections of the beet and by determination of the weight of the various sections, a calculation was made to obtain information regarding the weight, sugar content, and ash content of the remaining root that would be marketed by the different types of topping. This indicates that topping above the line *c* would increase the marketable weight of sugar beets and that such beets would have a slightly higher ash content and a slightly lower sugar content than

beets topped at the line *c* (Table 6), but the depreciation of sugar-beet quality which would arise from a change in practice to permit higher topping if either line *a* or line *b* were taken as a standard seems to be far less than the loss possible in storage piles where crown rot is serious.

TABLE 6.—*Comparison of weight, sugar, and ash content of differently topped sugar beets. Stored 84 days. Percentages computed in terms of beets topped in standard manner (Class c)*

Portion of beets (38 beet sample)	Total weight (ounces)	Weight	Sugar	Soluble ash	Insoluble ash	Total ash
		Per cent	Per cent	Per cent	Per cent	Per cent
Whole beets	1019	126.1	15.54	2.87	2.44	5.31
Root below line *a*	951	117.7	16.05	2.71	1.89	4.60
b	888	109.9	16.36	2.60	1.58	4.18
c	808	100.0	16.51	2.59	1.47	4.06
d	742	91.8	16.63	2.56	1.46	4.02
e	670	82.9	16.70	2.55	1.45	4.00

The present standards as to removal of crowns from beets have been based upon consideration of the condition of the beets at harvest time. The above tables indicate that under conditions where crown rot is prevalent during the storage period there is a benefit to be derived from leaving a part of the crown on the root. Since higher topping inhibits the development of crown rot to a marked degree in stored beets, sugar companies should consider carefully the savings possible by the practice of topping beets intended for storage higher than the present standard. The change in method of topping should not be made by any factory without a thorough study of local conditions as to seriousness of crown rot and as to effect of a change of practice on the quality of the beets. If crown rot is as serious as the writers' observations indicate, factories may find it to their advantage to secure beets that have been so topped as to leave part of the crown on the root.

DISCUSSION

At present, sugar beets are topped commercially at the base of the lowest leaf scar, in preparation for sugar extraction at the factory, regardless of whether they are to be used immediately or stored from 25 to 40 days or longer. Results of preliminary observations made at Lewiston, Utah,

and experimental work conducted in the field and laboratory at Logan, Utah, appear strongly to support the statement that, where sugar beets intended for storage purposes are topped at the base of the lowest leaf scar or lower, more beets show crown rot and the rot involves more tissue than when the beets are topped one-quarter to one-half inch above this region. In Cache Valley, Utah, more than 100,000 tons of beets were stored for an average period of 40 days. Experimental data obtained at Logan indicate that there may be from 10 to 15 per cent shrinkage of the beet substance suitable for sugar extraction from this one source, alone, where storage conditions are such that rot progresses strongly. It is believed that up to a certain degree, losses of the same character may occur fairly generally in commercial beet storage piles in the Western States. Since no survey has been made to establish loss figures other than in Cache Valley where 10 per cent of the crowns showed some degree of rot, one can only surmise what the actual loss may be in a season favoring storage rotting. Experimental consideration of this method of topping beets intended for storage by beet-sugar manufacturing companies may lead to changes in the present methods of topping of beets for storage and a partial saving of the present losses of sugar from stored beets.

Based on data collected in this study, approximately 7 per cent of the total number of beets milled are topped at *a*, or one-half inch above the base of the lowest leaf scar, and 27 per cent are topped at *b*, or one-quarter inch above the *c* region. Factory operations have not been impeded by the use of 34 per cent of the total number of beets milled which are topped at *a* and *b*. If all beets intended for storage were topped at *a* or even at *b* the amount of decayed tissue in the crowns which develops during storage would be decreased, while, on the other hand, there would be a greater tonnage for milling than where beets are topped at the line *c* or lower. From the data obtained, it is believed that, for stored beets where rotting is serious, the prevention of loss from rot which would come from leaving a higher crown on the beet would far more than offset the depreciation in quality.

Several theories have been considered as an explanation for the variation in the amount of decay in the crowns of stored beets. Morphologically, considerable difference is found in beet tissues in the five regions adopted for classification. In regions *a* and *b*, the fibrovascular bundles or rings are closer together and the amount of storage parenchyma is reduced. In the regions *c*, *d*, and *e*, parenchymatous tissues predominate, while the vascular rings are widely separated. Chemical analyses indicate a slight difference in percentage of sugar, purity, and ash content for the several regions. The organisms concerned in sugar-beet-crown decay are apparently better able to penetrate into spongy tissues where there is a high percentage of sugar (as in the case of beets topped at *c*, *d*, and *e*) than they

are in regions such as *a* and *b* where the spongy parenchyma is reduced, the sugar content is lower and the ash content is higher.

Undoubtedly studies on the rate of formation and the relative thickness of the corky layer formed on the cut surfaces of beets topped at different planes will prove to be important as means contributing to the final solution of the problem. Studies on these phases of the problem are now in progress.

SUMMARY

Isolation and inoculation studies indicate that *Phoma betae* is the most important pathogene concerned in the destruction of crown tissues of sugar beets under storage conditions, although two species of Fusarium are pathogenic to the sugar beet and may be of economic importance. All are wound parasites, but *P. betae* destroys crown tissues with greatest rapidity.

This paper constitutes a preliminary report on the correlation of storage losses in sugar beets with types of topping.

More than 10,000 beets were examined and classified as healthy or diseased. All beets were further classified according to the five divisions adopted for the description of the types of topping of commercial sugar beets.

Approximately 51 per cent of the total number of beets examined were topped at the base of the lowest leaf scar (*c*), or the commercial standard, 37 per cent of which were healthy and 63 per cent diseased. A marked decrease in numbers of diseased beets resulted when beets were topped one-fourth inch (*b*) and one-half inch (*a*) above this line, while a very decided increase in numbers of diseased beets occurred when the crowns were severed one-fourth inch (*d*) and one-half inch (*e*) below this line.

Where sugar beets were topped at the base of the lowest leaf scar (*c*) and lower (*d* and *e*), the amount of tissue which was decayed varied from 14 to 25 per cent; above this region (*a* and *b*) the amount of tissue which was decayed varied from 4 to 8 per cent.

Increased avoidance of attack by fungi was shown by stored beets topped higher on the crown than the topping practice recommended by commercial companies.

It is believed that sugar-beet companies could avoid a considerable part of the heavy loss from crown rot of stored beets by revision of the commercial standards for topping of the beets intended for storage.

OFFICE OF SUGAR PLANTS,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH
UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

INFECTION PHENOMENA AND HOST REACTIONS CAUSED BY TILLETIA TRITICI IN SUSCEPTIBLE AND NONSUSCEPTIBLE VARIETIES OF WHEAT[1]

H. M. WOOLMAN[2]

INTRODUCTION[3]

The investigation of the wheat-bunt problem of the Pacific Northwest, carried on by the Office of Cereal Crops and Diseases, in cooperation with the State experiment stations of Oregon and Washington, has shown conclusively that it can be solved most satisfactorily by the production of bunt-immune or highly resistant varieties. To eradicate bunt by this means, it is evident that varieties must be produced in which are combined the qualities of immunity from the bunt organisms, *Tilletia tritici* (Bjerk.) Wint. and *T. laevis* Kühn, and such other desirable characters as improved yield, strength of straw, and good milling and baking qualities. In other words, they must be at least as profitable when grown as are the susceptible varieties now in cultivation. In undertaking such a difficult problem, it seemed desirable first to have the fullest possible knowledge regarding all factors thought to influence resistance.

Observations made during several years' testing of varieties for bunt resistance have shown that the plants of many partially resistant varieties are able to suppress to a certain degree further invasion of their tissues by the fungus after infection takes place. This was indicated by the presence of both bunted and normal heads in stools of single plants at harvest time. The degree of smuttedness in these partially bunted plants varied from one grain of wheat in an otherwise totally bunted plant to the opposite extreme of one bunted grain in an otherwise bunt-free plant.

The purpose of these investigations, begun in 1919, was to determine, as far as possible, the nature of the factors underlying resistance. Are there varieties partially or totally resistant to the initial entrance of the bunt organism? In other words, is resistance due wholly or in part to the

[1] Cooperative investigations conducted by the Oregon Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] Formerly Field Assistant in Plant Pathology, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[3] The writer desires to express his obligations to Professor H. P. Barss, of the Oregon Agricultural College, for assistance in interpreting results of cytological slides; and to Dr. W. H. Tisdale, formerly of the Office of Cereal Crops and Diseases, and Dr. H. B. Humphrey, of the Office of Cereal Crops and Diseases, for assistance in preparing this manuscript.

structural nature of the epidermis? Or is it due wholly to the chemical nature of the cell contents? If the latter, at what stage in the development of the host plant do the inhibitive factors come into action?

WORK OF PREVIOUS INVESTIGATORS

So far as the writer has been able to ascertain, there is extant no literature dealing with the difference in reaction of the host to the bunt organism between susceptible and nonsusceptible varieties. As regards the mode of infection by *Tilletia tritici* or *T. laevis*, the development of the fungus within the host, and its physiological relations thereto, previous work is confined to Kühn, William Lang, Dastur, and Bolley. Brefeld and Wolf did not use Tilletia in their smut-infection studies.

The work of Kühn[4] is too well known to need comment, and to this day his conclusions remain unchallenged except, probably, in some particulars, by Lang. Kühn found the fungus hyphae to be partially intracellular and that they readily passed through the cell walls. Lang,[5] however, found them to be entirely intercellular. The writer frequently finds them to be intracellular in the coleoptile and the sheaths of the first and second true leaves and, therefore, in agreement with the observations of Kühn. He also finds that it is always intercellular in the nodes, internodes, and spike and in the blades of very young leaves, thus confirming Lang's observations.

The hyphae are often very difficult to find, owing to the fact stated by both Kühn and Lang that the protoplasm moves forward with the growth of the hypha, leaving a thin-wall, empty tube behind, which stains with difficulty and apparently is finally dissolved or digested by the plant juices and disappears entirely.

Bolley[6] made an extensive investigation of the relation of host to parasite in the mature plant but was led to erroneous conclusions, which fact he was the first to discover. Dastur[7] studied the entrance of the fungus to the epidermal cells and states that it gains entrance generally between the cells which it forces apart but that occasionally it passes directly through the epidermis into the lumina of the cells. He states that he obtained his infections by applying a culture of *T. tritici* spores to plants one day old, incubating them at 50° C. Judging from his figures, this abnormal

[4] Kühn, J. Die Krankheiten der Kulturgewächse, ihre Ursachen und ihre Verhütung. Aufl. 2, pp. 22, 312. 1859. Berlin.

[5] Lang, Wilhelm. Ueber die Beeinflussung der Wirtpflanze durch *Tilletia tritici*. Zeitschr. f. Pflanzenkrankh. 27: 80–99. 1917.

[6] Bolley, H. L. New studies upon the smut of wheat, oats, and barley with a resumé of treatment experiments for the last three years. N. Dak. Agr. Exp. Sta. Bul. 27: 109–164. 1897.

[7] Dastur, Jehangir Fardunji. Cytology of *Tilletia tritici* (Bjerk.) Wint. Ann. Bot. 35: 399–407. 1921.

temperature has produced infection that is morphologically very different from that obtained by the writer, whose cultures were maintained at 15° C., the optimum for the normal growth of the bunt organism.

A preliminary summary[8] of the results obtained by the writer has already been published.

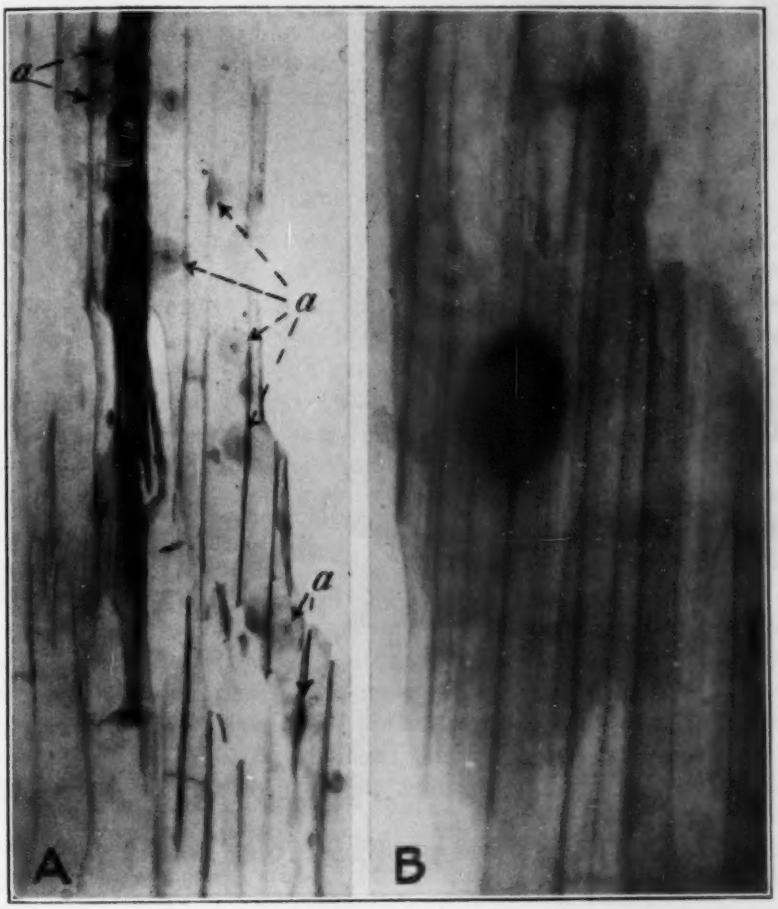

FIG. 1. A. A tangential surface section of the coleoptile from an 8-day plant of Hybrid 143 (susceptible), showing irregular grouping of points of attack, as at *a* (×200). Photograph by Dr. Erwin F. Smith. B. A tangential, near-surface section of the coleoptile from a Martin (nonsusceptible) plant about 7 days old, showing intense gram-positive area around point of entrance (×580). Photograph by Dr. Erwin F. Smith.

[8] Woolman, H. M. Cytological studies on the infection of wheat seedlings by *Tilletia tritici* (Bjerk.) Wint. (Abst.). Phytopath. **13**: 36–37. 1923.

Phases of Infection. For convenience, the development of the fungus within the host can be divided into three phases.

First: The entrance into and development within the epidermal layer of cells, during which time it is gram-negative in its staining reactions and both intercellular and intracellular in habit. (Figs. 1–5.)

Second: Its development in the deeper parts of the coleoptile and in the sheaths of the true leaves, especially the first and second leaves, where it is gram-positive and both intercellular and intracellular. (Fig. 6.)

Third: Its development in the very young leaf blades, the nodes, internodes, and the growing point, where it is gram-positive and strictly intercellular. (Fig. 7.)

INVESTIGATIONS

The work in 1919 to 1920, inclusive, was performed under great difficulties, due in part to inadequate equipment and in part to the writer's own shortcomings. The thermostat, supposed to control the hot-air oven in which the embedding was done, frequently failed to control the temperature, and, as it was impossible to keep a day and night watch, several lots of plants were lost from overheating. Others were so overhardened that parts were lost in the process of cutting, fixing, and staining. As a consequence of the foregoing facts and the faulty technique, the work from 1919 to 1920, inclusive, deals only with the second and third phases of infection. The first or entrance phase was not observed.

Method of obtaining infected plants. A mass of steam-sterilized soil was air-dried and mixed with 2 per cent of its weight of crushed bunt balls. This mixture was then divided into four equal parts, each part in succession being well moistened at two-day intervals. When the last part was moistened, all were thoroughly mixed together and the seed planted at a uniform depth of 5.5 cm. At this time, the spores in the portion first moistened had germinated and were producing conidia profusely. Thus the presence of an active and virulent inoculum was insured for several days after planting.

During the entire time from preparation of the infested soil until the last plants were removed for killing, the operations were conducted in a greenhouse maintained at approximately 15° C.

Killing and staining technique. The plants were killed in a mixture of equal parts of absolute alcohol and glacial acetic acid. They were then run through absolute alcohol to xylol and embedded in 60° paraffin.

The fungal hyphae are generally gram-positive in their staining reactions when within the host. Therefore, gentian-violet, followed by Gram's iodine solution, was used as a differential stain. Orange G or eosin was employed as a counter stain. However, at its initial entrance into the epidermal

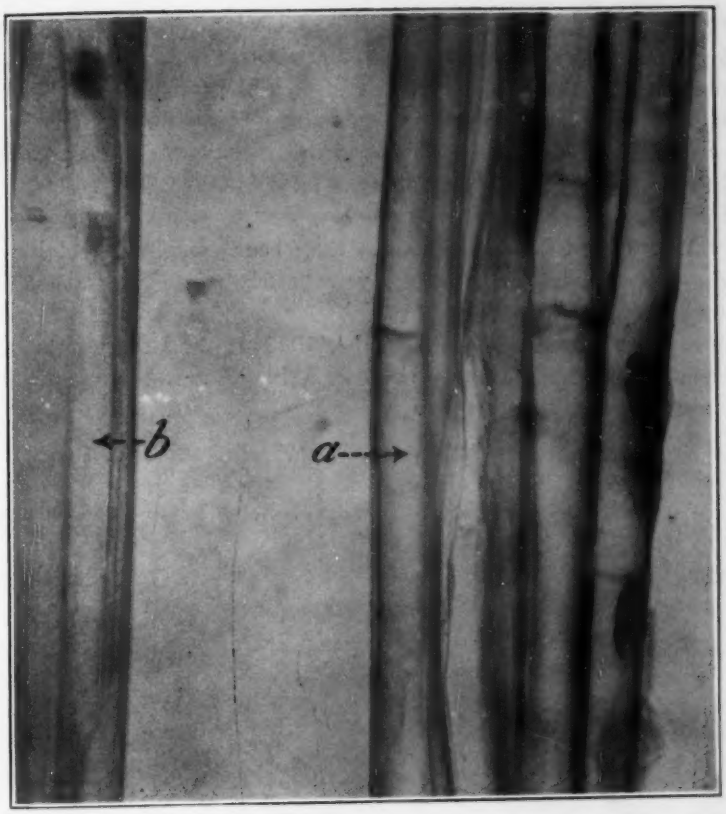

FIG. 2. A radial section from a Martin (nonsusceptible) plant about 7 days old, showing early stage of infection at *a* and wide interspace between coleoptile and *b* sheath of first true leaf (×525). Photograph by Dr. Erwin F. Smith.

cells the invading fungus seems to be gram-negative, as well as inert to both orange G and eosin. But at this stage the cell wall, the middle lamella, and the cytoplasm in the immediate vicinity of the point of entrance are decidedly gram-positive. Owing to this difference in standing reaction, the points of entrance were not recognized in the examinations made from 1919 to 1921, the hyphae being completely obscured by the dense violet color in the surrounding tissue. The notes on infection during the first two years' work, therefore, refer only to the second and third phases of development, in which the mycelium takes the violet stain and the surrounding tissue or cytoplasm reacts to the counter stain only. Therefore, it can not be assumed that when plants are recorded as noninfected no entrance of the fungus has taken place.

Hybrid 143, an extremely susceptible club wheat, Turkey Wn. 326, and Florence, moderately resistant, and two selections from Turkey Wn. 326 x Florence, produced by E. F. Gaines, of Washington State College, were studied for differences in resistance to bunt. The two selections from the Turkey and Florence cross showed results in many field tests indicating that they were much more resistant than either parent.

To eliminate to the utmost the errors due to small numbers, the two Turkey Wn. 326 x Florence selections have been combined in table 1 and treated as one variety, the two being very closely related and about equally resistant to bunt.

The sowings were made on November 10, 1919, and the plants emerged on November 20. Killings were made 6, 9, 16, 32, and 60 days after emer-

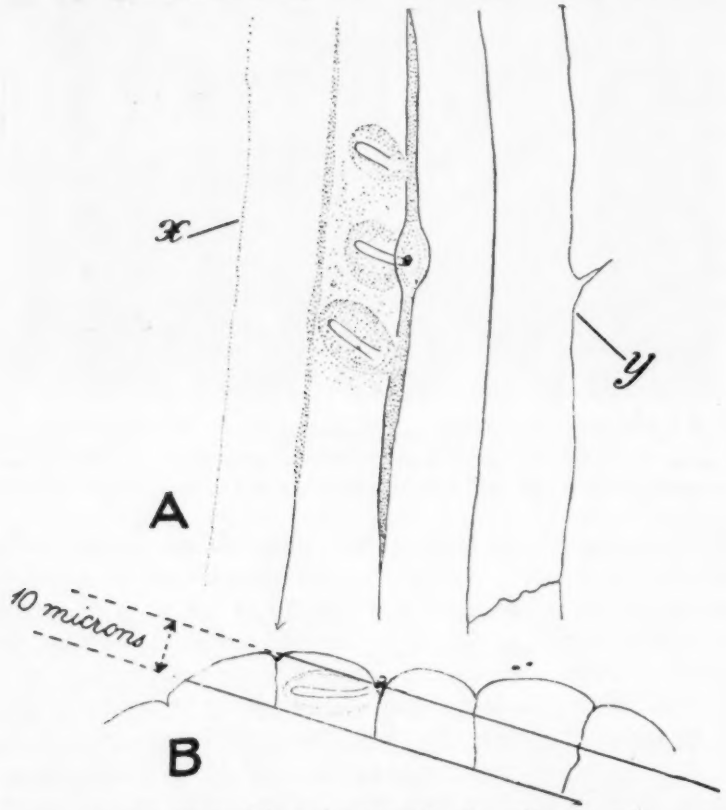

FIG. 3. A. A near-tangential section through the epidermal cells of a Hussar (highly resistant) plant about 7 days old, showing entrance of hyphae between walls of contiguous epidermal cells of the coleoptile and thence passing into the lumen. (Camera-lucida drawing, × 600.) B. A theoretical cross section of figure A on line *x-y*, showing point of entry of infecting hypha.

TABLE 1.—*Results of cytological studies showing percentages of plants found to be infected at definite periods of time after emergence, Corvallis, Oreg.*

Variety	Age of plant									
	6 days		9 days		16 days		32 days		60 days	
	+[a]	−[b]	+	−	+	−	+	−	+	−
Hybrid 143	15	10	8	8	5	5	8	0	16	9
Turkey Wn. 326	3	21	4	8	Lost		Lost		Lost	
Florence	0	25	4	20	Lost		12	0	0	10
Turkey Wn. 326 × Florence selections	8	17	4	16	15	9	7	16	2	9

[a] + indicates infection found.
[b] − indicates infection not found.

TABLE 2.—*Results obtained from plants grown to maturity in the greenhouse from the same sowings recorded in table 1*

Variety	Number of plants		
	Bunt-free	Partially bunted	Totally bunted
Hybrid 143	2	0	8
Turkey Wn. 326	9	0	1
Florence	8	0	0
Turkey Wn. 326 × Florence selections	55	0	0

TABLE 3.—*Results obtained from plants transplanted to field from sowings recorded in table 1*

Variety	Number of plants		
	Bunt-free	Partially bunted	Totally bunted
Hybrid 143	0	0	24
Turkey Wn. 326 × Florence selections	52	8	3

gence. Unfortunately, several of these lots were lost and others more or less damaged during the process of embedding, so that the record of examination is incomplete. Therefore, any attempt to draw definite conclusions from the percentage figure alone would be unsafe. Also, a wide margin should be allowed for experimental error in examination; that is, a failure to find a few bits of small faintly stained mycelium in the great mass of tissue searched.

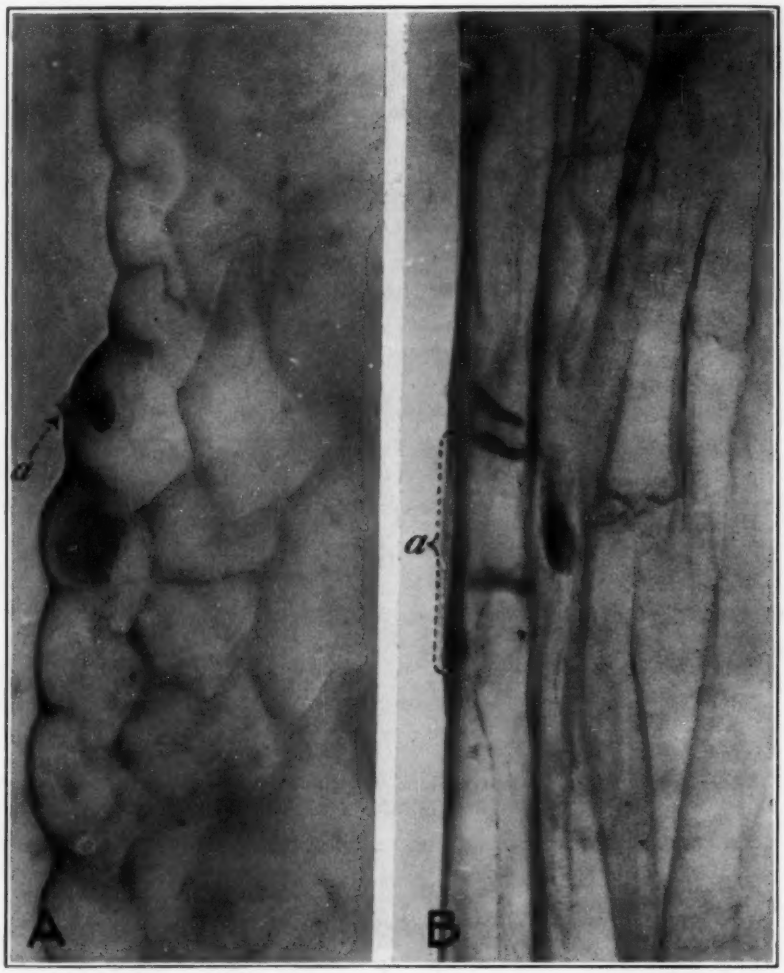

FIG. 4. A. A cross section of a Hussar (highly resistant) plant about 7 days old, showing characteristic thickening of epidermal wall at entrance and external hypha *a* (× 810). Photograph by Dr. Erwin F. Smith. B. A radial section from a Hussar plant about 7 days old, showing the sheaths enclosing the infecting hypha in the epidermal cell. An external hypha *a* is also shown. (× 810.) Photograph by Dr. Erwin F. Smith.

OBSERVATIONS ON NATURE OF INFECTION

The appearance of the infection in susceptible and nonsusceptible plants killed when 6 days old was in every way similar in both location and development. In all cases the fungus was confined to the coleoptile. The fungus also was confined to the coleoptile in seedlings of resistant varieties killed at 9 days of age, except in rare cases. In the case of Hybrid 143 it frequently had entered the first true leaf. When 16 days old the difference between the two classes is very apparent, both with reference to amount and location of the mycelium. In the Turkey Wn. 326 × Florence selections it had advanced in no case further than the contact between the sheaths of the first and second leaves and was not common; while in Hybrid 143 it was in many cases profuse and had penetrated into the second leaf sheath and into the subsurface internode. When 32 days old, the differences were still more pronounced, and, when 60 days old, the Turkey Wn. 326 × Florence selections, when infection was found at all, as a general rule, showed only small, slightly infected areas, which were seldom deeper than the third leaf. Plants from this sowing grown to maturity showed about the same percentage of infection as was found in the 60-day-old plants. In Hybrid 143, at the age of 60 days, the mycelium was found to be extensively and profusely distributed through the tissues of all the leaves and in the axis of the plant, and in some cases it had reached the growing point.

Apparently in the resistant variety, the suppressive action begins about the time the mycelium passes into the first true leaves or soon after the plant is 9 days old; and, except in rare instances, it ceases to be a menace to the plant by the time it is 60 days old.

Hybrid 143, Hussar (C. I. 4843), and Martin (C. I. 4463) were employed in the studies begun in 1921. The first variety is highly susceptible and the other two are apparently immune, as they had previously failed to produce even a trace of bunt in repeated field tests. Sowings were made in infested soil, as previously described, at a depth of 5.5 cm. The seedlings were killed for examination at the time of emergence and 7, 12, and 20[9] days thereafter. The method of staining was the same as that outlined in the foregoing pages except that the intensity of the gentian-violet-gram stain was reduced and the sections cut to a thickness of not more than 10 microns and generally as thin as 6 microns.

The examination of 60 plants of Martin, 40 of Hussar, and 20 of Hybrid 143 showed that the fungus entered the epidermis of the seedlings of both susceptible and resistant varieties apparently with equal facility. In all plants killed when 7 days old numerous points of entrance were found. As a general rule, no fewer than 100 points of attack per plant would be found.

[9] Ages only approximate, as plants vary at least 24 hours in emergence.

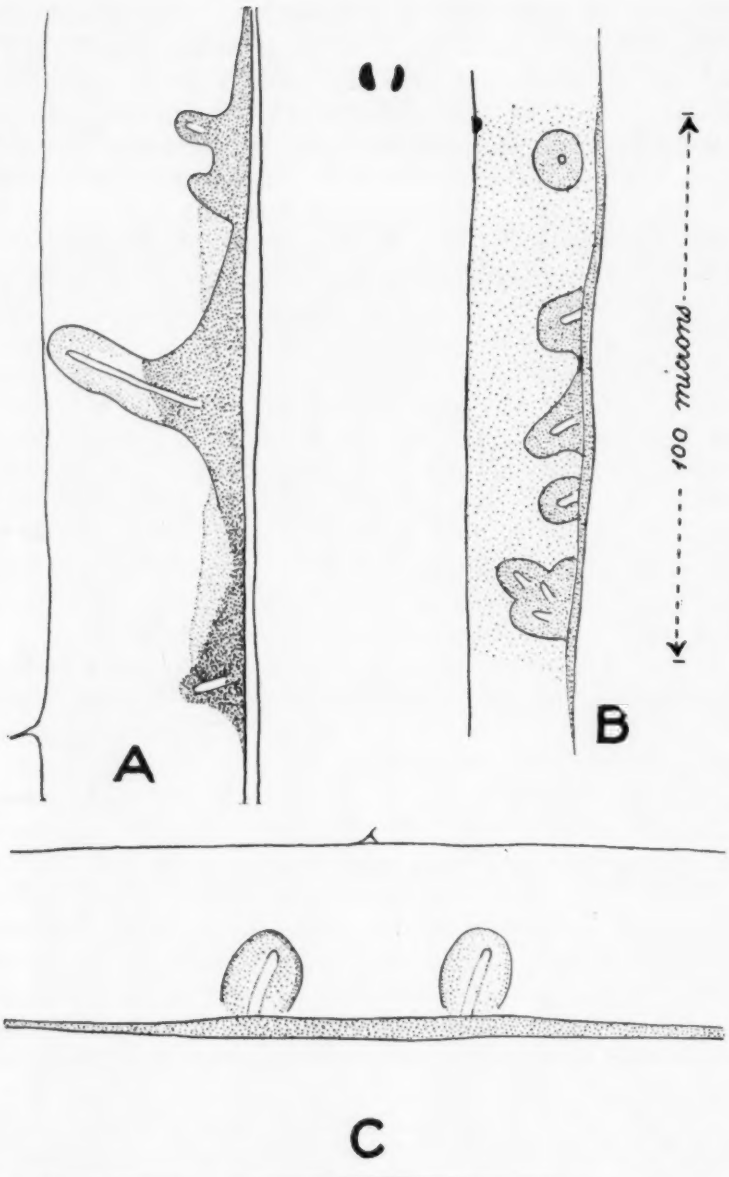

FIG. 5. Near-radial sections from Hussar plants about 7 days old, showing hyphae with enclosing sheaths in epidermal cells of coleoptile. (Camera-lucida drawings: A, × 1,000; B, × 730; C, × 800.)

In plants killed at the time of emergence but few points or groups of points of attack, usually 2 or 3 per plant, could be seen. But no plant was found to be entirely free. Evidently infection begins at about the time of emergence. This, however, may not be true for all environmental conditions of soil moisture, temperature, etc. In 25 per cent of the plants of Hybrid 143, killed when 7 days old, the second phase of infection was found. In Hussar and Martin, the second phase was found in only 5 per cent of all plants examined up to 20 days of age. In fact, so far as these examinations have progressed, it seems that in these two resistant varieties, the infection rarely develops beyond the first phase. No difference could be detected between plants 7 and 20 days old. It seems, therefore, that an inhibiting factor comes into action as soon as the hypha enters the lumen of an epidermal cell of the coleoptile. There is some reason to believe that even the most susceptible varieties possess, in some degree, such an inhibiting factor. This is suggested by the fact that in 70 plants of Hybrid 143 examined from 1919 to 1923 between the ages of 7 and 20 days never more than four distinct areas of second-phase infection have been found in the same plant and rarely more than two. These areas were in most cases sufficiently distinct to indicate that they had developed from separate entrances. The absence of the second phase of infection in other parts of the plant seems to indicate that at many points of entrance the fungus had failed to develop this phase. It, therefore, seems that some inhibiting factor or factors have prevented the development of the second phase. What these inhibiting factors are is as yet an unsolved problem.

TABLE 4.—*Results of cytological studies of wheat plants grown in bunt-inoculated soil and killed at definite ages, 1921 to 1923, Corvallis, Oreg.*

Variety	Age of plants when killed			
	At emergence	7 days	12 days	20 days
Wn. Hybrid 143	Five plants examined; 1st phase infection found in all; 2nd and 3rd found in none	Eight plants examined; 1st phase found in all; 2nd phase in 2; 3rd phase in none	Seven plants examined; all phases found in all plants	None examined
Martin, C. I. 4463	Fifteen plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Fifteen plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Fifteen plants examined; 1st phase found in all; 2nd phase in 2; 3rd phase in none	Fifteen plants examined; 1st phase found in all; 2nd phase in 1; 3rd phase in none
Hussar, C. I. 4843	Ten plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Ten plants examined; 1st phase found in all; 2nd and 3rd phases found in none	Ten plants examined; 1st phase found in all; 2nd phase found in 1; third phase found in none	Ten plants examined; 1st phase found in all; 2nd phase found in 1; 3rd phase found in none

Location of Entrance.[10] About 75 per cent of the points of attack are found within 2.5 cm. above the seed from which the plant grew, notwithstanding the fact that the soil was evenly infested from the seed to the surface.

Entrance Phenomena. As has been stated by Dastur, the infecting hypha generally enters between the vertical walls of the epidermal

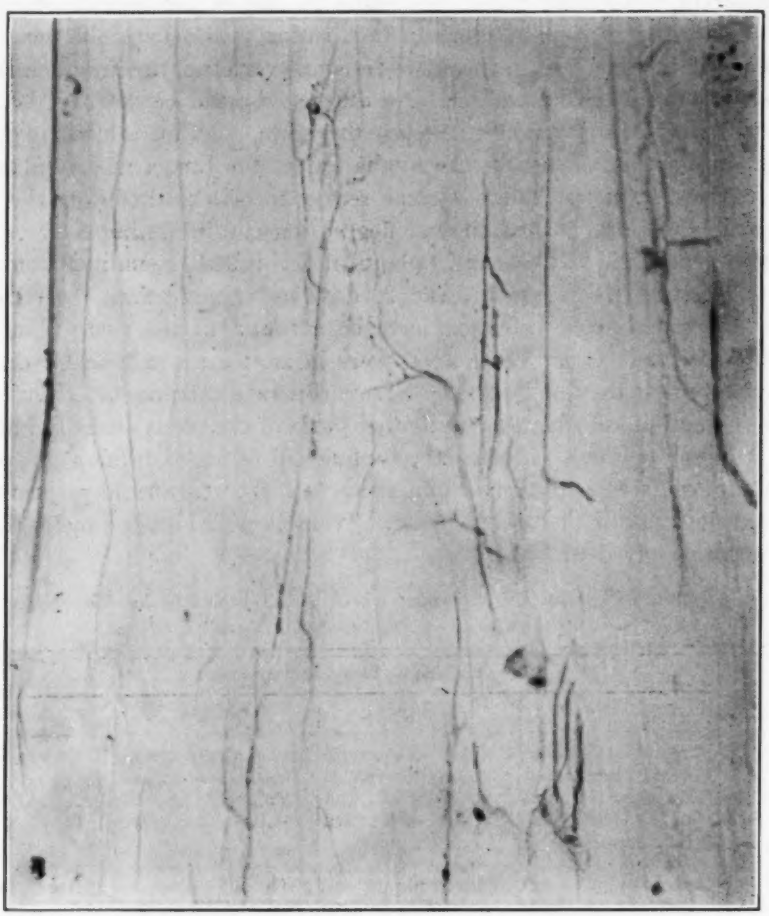

FIG. 6. A tangential section through coleoptile of a Hybrid 143 (susceptible) plant 15 days old, showing profuse second-phase infection (× 520).
Photograph by W. C. Mathews.

[10] Previous experiments have shown that wheat plants can be grown through 1½ inches of sterile soil into an infested soil and yet produce bunted heads. That is, effective infection can occur more than 1½ inches above the seed. It also was found that plants can be grown in sterilized soil until the second true leaf appears and then become infected by transferring to infested soil.

cells, but occasionally it passes directly through the epidermis into the lumen of the cell. This can be demonstrated in cross sections (Fig. 4, A).

It is the opinion of the author that, after penetrating between the cells, the hypha usually enters the lumen of one of the adjacent cells (Fig. 3). This point, however, is sometimes hard to demonstrate in longitudinal sections, and sufficient examinations in cross sections have not been made to justify definite conclusions.

The first indication of an attack along the cell wall, when viewed in a longitudinal radial section, is a thickening of the epidermis for a distance of 20 or more microns which is highly gram-positive. When seen in tangential section, it appears as a circular spot which sometimes exhibits zonation. This thickening is apparently due to gelatinization of the portion of the epidermis lying between the cuticle and the plasma membrane (Figs. 1, 2, 3, and 4, A). A little later a conical protuberance is seen on the inside of the epidermis just beneath the point of attack by the hypha. In some cases this conical portion appears to be an indentation of the epidermal wall, as if the wall had yielded to mechanical pressure. However, this probably is not the case, as in later stages the hypha is seen to pass through the wall without any displacing effect. On the other hand, the entry of the hypha between cell walls, as seen in a tangential section, does produce a lateral displacement (Fig. 3). With the growth of the hypha this conical protuberance becomes, first, more or less globular (Figs. 2 and 4, A) and, finally, an elongated cylindrical sheath of from 8 to 12 microns in diameter enclosing the hypha, which has a diameter of 1 micron or less (Fig. 4, B). This sheath is probably what Wolf[11] observed in his studies of infection by *Urocystis occulta* (Wal.) Rab. and called a cellulose sheath. No photomicrograph has yet been obtained which shows the actual hypha within the sheath. It can, however, be seen plainly under the microscope (Figs. 3 and 5).

No adequate investigation of the physical or chemical nature of this sheath has been made, but, because of its intensely gram-positive reaction, it apparently is not cellulose. Usually, though not always, the space enclosed by it seems to take a deeper violet than the surrounding space, but this appearance probably is deceptive and the apparent color due to the fact that the interior is seen either through the sheath or against it as a background in the very thin microtome sections. Invariably there is a gram-positive condition at the point of entrance and its immediate vicinity.

There is apparently a decided tendency for points of penetration to appear in groups. Sometimes they are scattered irregularly over an area of 1 to 3 sq. mm., as seen in figure 1, A, but more often they are found at close

[11] Wolf, R. Der Brand des Getreides sein Ursachen und Verhütung. Halle, 1874.

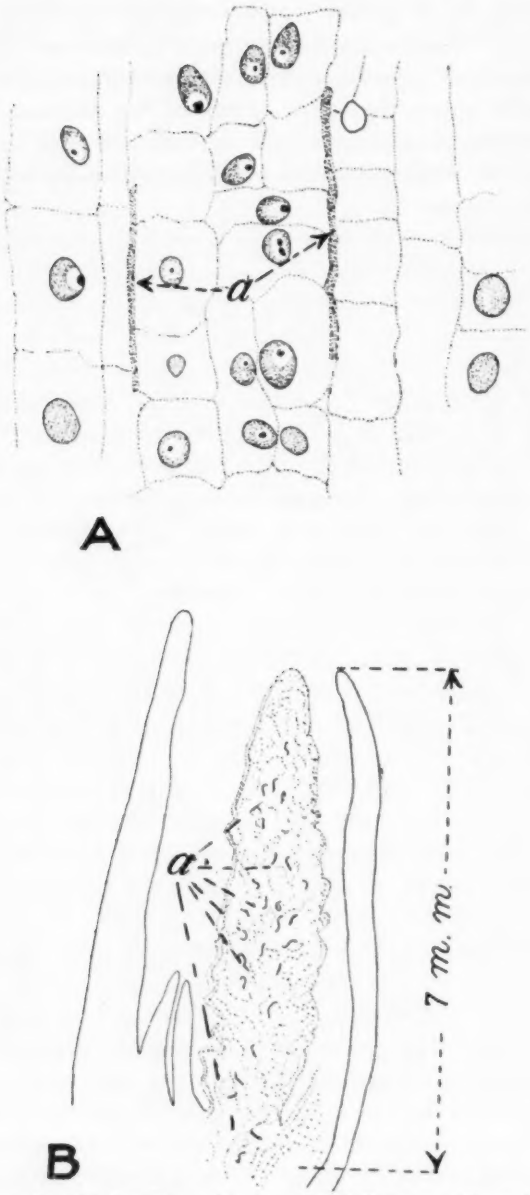

FIG. 7. Camera-lucida drawings showing third-phase mycelium in a Hybrid 143 (susceptible) plant 90 days old. A. Intercellular hyphae at *a* in the blade of one of the youngest leaves. Note normal appearance of the host nuclei. (×1,000.) B. Section through the embryonic head of the same plant (×100). *a* Indicates fragments of mycelium of which 25 were counted in this section.

intervals along the vertical cell walls, where the entering hyphae apparently spring from an external hypha lying along a cell-wall depression of the coleoptile (Fig. 5). An external hypha is seen in figure 4, B, and in cross sections in figure 4, A, but in this case it is between depressions.

The transition from the first to the second phase of infection is as yet obscure. The second phase in which the hypha becomes gram-positive is very rarely found in the epidermal cells, but frequently is found in the second cell layer near the points of entrance. The actual change has not yet been observed.

It seems a reasonable assumption that the change from first to second phase, that is to say, from gram-negative to gram-positive, is coincident with its establishing parasitic relations with the host.

Many questions regarding the process of infection and related phenomena remain for investigation, such, for example, as the chemical changes within the host and the influence of such environmental conditions as temperature and moisture in proximity to the seedling, pH concentration of the soil, and the presence or absence of toxic inhibitive substances or organisms resident within the soil. On these questions various hypotheses suggest themselves, but the object of this paper is to state observed facts rather than to indulge in speculation. The investigation should be continued. Its continuation by the writer was prevented by his retirement on account of age. It is now published in the hope that it may be a help to some younger investigator. The writer will gladly answer, in so far as possible, any inquiries from any one pursuing similar research.

SUMMARY

The process of infection of wheat seedlings by *Tilletia tritici* can be divided into three phases.

First: The entrance of the hypha into and its development within the epidermal cell, in which phase it is gram-negative and both intercellular and intracellular.

Second: Its development in the deeper parts of the coleoptile and in the sheath tissues of the earliest true leaves. In this phase, it is gram-positive and both intercellular and intracellular.

Third: Its development in the very young leaf blades and in the nodes, internodes, and the growing points of the plant, where it is gram-positive and strictly intercellular.

The fungus enters seedlings of both resistant and susceptible varieties of wheat with equal facility, but, in the most resistant varieties studied, apparently it does not develop beyond the first phase above mentioned, except in rare instances.

In varieties a little less resistant, the fungus develops the second phase of infection about as readily as it does in the susceptible varieties until the seedling is about 9 days old, after which it is retarded, and, in a high percentage of cases, ceases to be a menace to its host by the time it is 60 days old. In all varieties thus far examined, the entrance of the hyphae produces a gram-positive condition in the cell walls and cytoplasm in the immediate vicinity of the points of penetration.

TWO HARBORS,
MINNESOTA.

BACTERIAL SPOT OF RADISH AND TURNIP[1]

HAROLD E. WHITE[2]

INTRODUCTION

A previously undescribed bacterial-spot disease of radish and turnip has been found in Indiana (10). It is caused by a yellow monoflagellate bacterium which, in inoculation tests, has proved pathogenic not only to radish, turnip, and a number of other cruciferous plants, including cabbage, kale, cauliflower, and Brussels sprouts, but also to tomato, tobacco, and pepper. Its host range and cultural similarity to *Bacterium campestre*, *Bact. campestre* var. *armoraceae*, and *Bact. vesicatorium* raised the question of its relationship to these organisms. The radish and turnip organism is tentatively designated herein as *Bact. vesicatorium* var. *raphani*. A description of the symptoms produced on the various hosts, a greenhouse inoculation study of its pathogenicity, a comparative cultural study of the causal organism, and proof of its transmission with radish seed are reported in this paper.

The bacterial-spot disease of turnips was found by M. W. Gardner at Matthews, Indiana, in June, 1928. In July the writer found a similar disease on radishes growing in a greenhouse at Purdue University. The radishes were so heavily infected that the leaves were being rapidly killed by petiole lesions. In August, 1925, Gardner (2) found a disease near Indianapolis, Indiana, causing small black lesions on radish leaves, stems, and seed pods. Although an Alternaria was associated with these lesions, the symptoms of this disease agreed closely with those of the bacterial spot of radish, and very likely it was the same disease. A search of the literature has not revealed any previous description of a bacterial-spot disease of radish or turnip caused by a yellow organism.

CAUSAL ORGANISM ISOLATED FROM RADISH AND TURNIP

Isolations were made by macerating a portion of a lesion in sterile water on a flamed slide and plating out from this water in potato-dextrose agar by the loop-dilution method. Isolations were made from spots on leaves of turnip collected at Matthews, Indiana, and from radish at La Fayette,

[1] Contribution from the Botany Department, Purdue University Agricultural Experiment Station, La Fayette, Indiana. A thesis presented to the faculty of the Graduate School of Purdue University in partial fulfillment of the requirements for the degree of Master of Science.

[2] The writer wishes to acknowledge his indebtedness to Prof. M. W. Gardner for suggestions and guidance in the development of this investigation and to Prof. E. J. Kohl and Mr. R. W. Samson for assistance.

Indiana. From these two sources and from all types of lesions the same type of yellow, rapidly growing bacterial colony was obtained. The pathogenicity of these two strains was proved by inoculation and reisolation and the purity of the cultures was proved by poured plates. No marked differences were noted between the two strains. Morphological, physiological, and cross-inoculation studies proved that the two strains were identical and either strain may be subsequently referred to as the radish organism. Greenhouse inoculations, to be described later, showed that mustard, cabbage, kale, Brussels sprouts, cauliflower, tobacco, pepper, and tomato plants were also infected by this organism.

SYMPTOMS ON RADISH, TURNIP, AND OTHER CRUCIFERS

In greenhouse-inoculation tests, the spots on radish leaves are irregularly circular, and are 2 to 3 mm. broad, sometimes larger. Very young

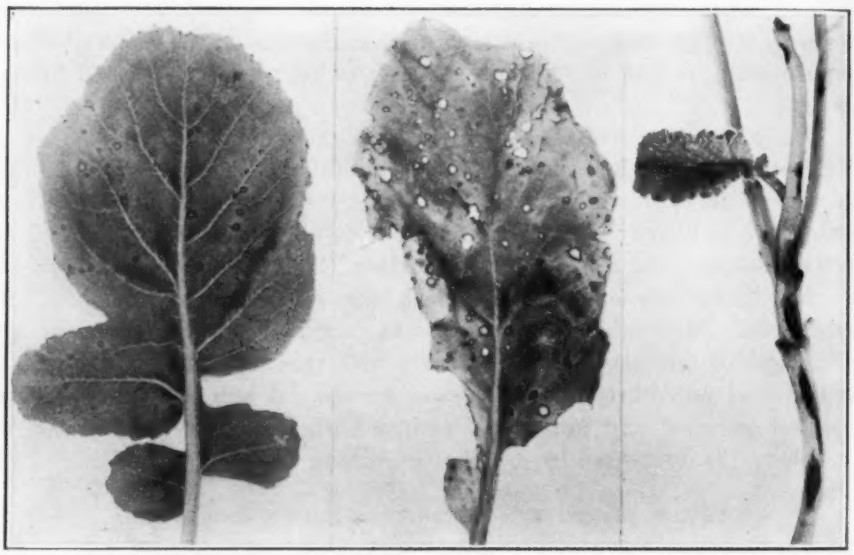

FIG. 1. Circular bacterial-spot lesions on radish leaves and elongated, sunken, stem and petiole lesions on radish seedling as a result of atomizer inoculation in the greenhouse.

spots appear as small circular black dots, later becoming light brown (Fig. 1). The larger lesions are light tan, sunken, slightly glazed, and surrounded by a narrow, yellowish, water-soaked zone, visible only upon close observation. The spots on the leaves frequently coalesce and become irregular in form but are not limited by the veins. Where the lesions are

numerous along the veins and midrib of the growing leaf, considerable distortion of the leaf blade occurs.

Petiole and stem lesions on radish are black, sunken, elongated, and 5 to 10 mm. long (Fig. 1). As the lesion increases in size it penetrates rather deeply and the petiole often breaks off at the point of infection. Petiole infection on young plants is the most destructive effect of this disease and with heavy infection an entire plant may be destroyed within 10 to 14 days after symptoms appear. Cotyledon lesions occur abundantly upon inoculated radish seedlings and plants from infested seeds. The cotyledon lesions are definitely circular in shape, 3 to 4 mm. in diameter, sunken, dark brown and water-soaked (Fig. 2, C).

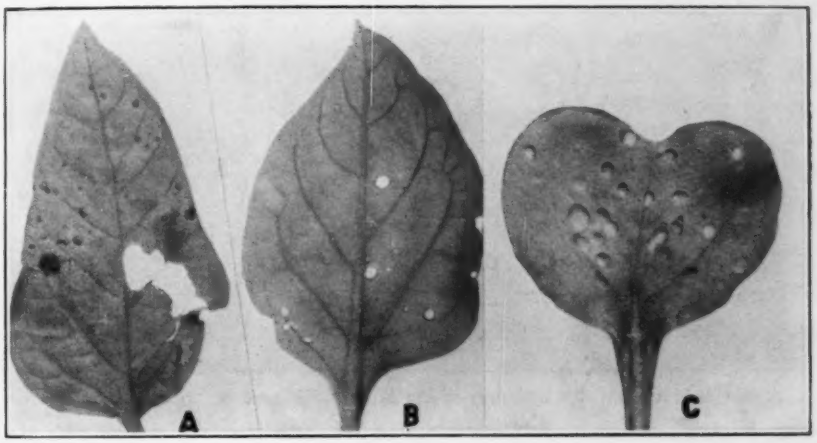

FIG. 2. A. Pepper leaf showing black lesions caused by *Bact. vesicatorium* from tomato. B. Pepper leaf showing circular whitish lesions caused by radish organism. C. Radish cotyledon showing circular lesions resulting from atomizer inoculation.

The lesions on turnip leaves and petioles are of the same general nature as those on radish. Cotyledon infection also occurs on turnip. The lesions on mustard leaves are at first small, black, circular spots, later 2 to 3 mm. in diameter, circular, greasy brown, and finally light tan. Petiole lesions are of the same nature as those on radish and are equally destructive on young plants.

The lesions on the leaves of cabbage, cauliflower, Brussels sprouts, and kale first appear as depressed circular black specks with water-soaked margins. As they increase in size the lesions remain circular and become more noticeably sunken, with a pale yellowish halo a millimeter or more in width. The lesions range from 2 to 4 mm. in diameter and, with age, change from a tan color in the center, surrounded by a purplish margin, to a chalky white. This chalky whiteness and the circular shape are the most striking

features of the lesions occurring on these hosts (Fig. 3). The petiole lesions on young plants of these hosts resemble those occurring on radish and turnip, and cotyledon infection is common. The petiole lesions do not occur so abundantly, however, and the disease is not so destructive on these hosts as on radish, turnip, or mustard plants.

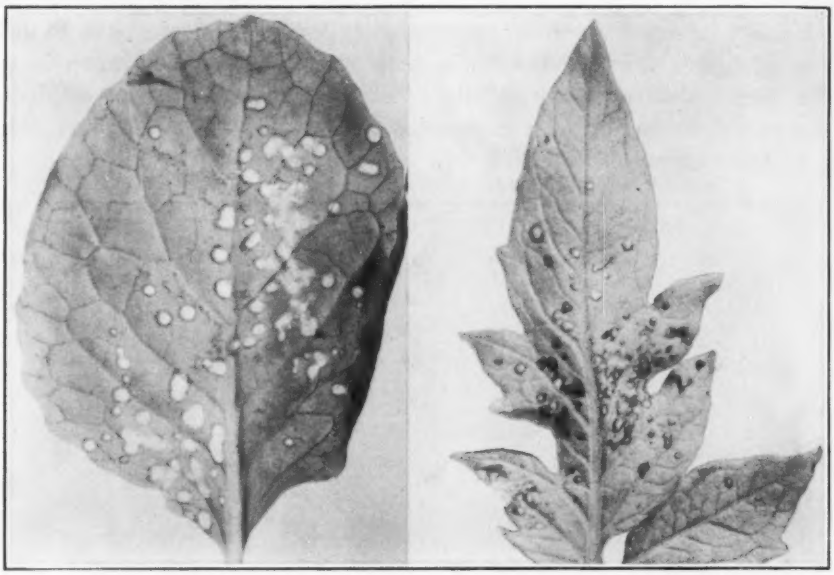

FIG. 3. Lesions caused by atomizer inoculation with radish organism on leaves of cabbage and tomato seedlings.

SYMPTOMS ON TOBACCO, PEPPER, AND TOMATO

In greenhouse-inoculation tests the leaf lesions on tobacco plants are at first small, black, sunken spots, later chalky white with a slightly water-soaked border, and seldom more than 2 mm. in diameter when mature. Petiole infection was not observed.

Lesions on leaves of pepper plants are at first small, black, circular, sunken, water-soaked areas, becoming 3 to 4 mm. in diameter, dark brown, and later fading from brown to a chalky white (Fig. 2 B).

The lesions on tomato leaves first appear as circular black spots, later becoming 3 to 4 mm. in diameter, with light tan centers surrounded by a dark brown zone and, around this, a water-soaked margin (Fig. 3). Petiole and stem lesions on tomato are elliptical or irregular in shape, sunken, at first light brown, later black, and 4 to 8 mm. long. In the seedling stage the lesions often cut off the leaf or stem at the point of infection. Fruit lesions obtained by needle-prick inoculation are circular dark brown or black sunken areas about 5 to 10 mm. in diameter. The fruit tissue is invaded and blackened to a considerable depth under the lesion.

PATHOGENICITY OF CAUSAL ORGANISM

By means of inoculation tests, information was obtained concerning incubation period, mode of entry into host tissues, relationship to *Bact. campestre* and *Bact. vesicatorium*, host range, and varietal susceptibility. Cultures from two sources were used in inoculation work, one obtained from turnip at Matthews, Ind., and the other from radish at La Fayette, Ind. Results were confirmed by reisolation of the organism.

Incubation period and mode of entry. Leaf and petiole infection was readily obtained in the greenhouse by spraying very young radish and turnip plants with an atomizer containing a water suspension of the bacteria. The plants, after inoculation, were held in a moist chamber for 24 to 36 hours. In summer, lesions were visible within 4 to 5 days, but in winter, under greenhouse conditions, the inoculation period was as much as 10 to 15 days. The abundance of lesions obtained by atomizer inoculation indicated that wounds were not necessary for infection and that the mode of entry into the host tissues was through the stomata. Marginal leaf lesions occurred, probably as a result of hydathode infection, but were localized, and isolations made from tissue taken about one millimeter distant, along the veins, from the inner edge of such marginal lesions yielded no colonies and showed that vascular invasion had not occurred. The control plants, sprayed with water, remained free from infection.

Host range. In August, cabbage, cauliflower, kale, Brussels sprouts, and mustard were inoculated with the two strains of the radish organism by spraying with an atomizer. Five days from the date of inoculation lesions appeared on all the inoculated plants. Lesions occurred along the leaf margins, but there was no vascular invasion such as is characteristic of *Bact. campestre*. The noninoculated control plants of each host remained free from infection. Infection was obtained also on tomato, pepper, and tobacco plants in the greenhouse. Unsuccessful attempts were made to infect garden beans, soybeans, horse-radish, nasturtium, garden cress, shepherd's purse, and garden huckleberry (*Physalis* sp.).

Comparison with Bact. campestre and Bact. vesicatorium. Since the host range of the radish organism included hosts of two yellow organisms, *Bact. campestre*, the cause of black rot of crucifers, and *Bact. vesicatorium*, the cause of bacterial spot of tomato and pepper, it was necessary to compare the three organisms by inoculation as well as cultural tests. A culture of *Bact. campestre* was obtained from the University of Wisconsin and a culture of *Bact. vesicatorium* was isolated from tomato fruit. These two organisms and the radish organism were tested in a parallel series of inoculations on radish, turnip, mustard, cabbage, cauliflower, kale, Brussels sprouts, tomato, pepper, and tobacco. Infection took place on all the hosts

with the two strains of the radish organism. Vascular infection on cabbage was obtained with *Bact. campestre,* but no infection occurred on any of the other host plants. *Bacterium vesicatorium* infected pepper and tomato but none of the other plants. Under the conditions of these tests, the radish organism was pathogenic to a wider range of hosts than either of the other organisms.

Parallel inoculations were made on green tomato fruits with *Bact. vesicatorium* and the radish organism. Green tomatoes, gathered in the greenhouse, were washed with mercuric chloride solution, 1 to 1000, then with sterile water, and placed in sterile moist chambers. Punctures were made in the tomato fruits with a flamed needle dipped in a water suspension of the bacterial culture. The moist chambers with the inoculated fruits were kept at a temperature of 21° C. Seven to ten days after inoculation the fruits inoculated with the radish organism showed circular, sunken, black lesions around the punctures, 4 to 5 mm. in diameter, with an outer water-soaked zone often surrounded by a yellow halo. The inoculations with *Bact. vesicatorium* caused lesions closely resembling those formed by the radish organism.

Varietal susceptibility. A total of 42 seedsman varieties of radishes have been tested and all proved susceptible to infection. Likewise 9 varieties of turnips, 7 varieties of kale, 5 varieties of Brussels sprouts, 6 varieties of mustard, 6 varieties of cabbage, and 3 varieties of cauliflower were infected. No resistant varieties of any of these hosts were found.

MORPHOLOGY AND CULTURAL CHARACTERS OF THE CAUSAL ORGANISM

The organism is rod-shape and is motile by means of one polar flagellum. When stained with Van Ermengem's flagella stain, the cells appeared to have a thin capsule and varied in width from 0.65 to 1.2 μ and in length from 2.0 to 3.1 μ with an average of 0.96 by 2.49 μ. This is slightly in excess of the average length (1.94 μ) of the cells of *Bact. vesicatorium* (3). Otherwise the similarity is very close. Endospores and involution forms have not been observed.

The organism grows very readily on potato agar containing 2 per cent dextrose and no peptone and less vigorously on corn-meal agar or beef-peptone agar. The organism was grown in parallel series of cultures with *Bact. vesicatorium, Bact. campestre,* and *B. coli* on beef-peptone agar, potato-dextrose agar, gelatin, potato cylinders, milk, litmus milk, milk with brom cresol purple, milk with phenol red, nitrate bouillon, 2 per cent peptone solutions containing the different carbon sources, dextrose, saccharose, maltose, lactose, glycerin and mannit, nutrient agar containing these different carbon sources and the H-ion indicators, phenol red, brom thymol

blue, and brom cresol purple, nutrient agar with starch, Uschinsky's, Fermi's, and Cohn's solutions, blood serum, and sodium chloride solutions.

Gelatin was liquefied. No acid was produced in milk and no acid or gas was produced with the different carbon sources. Starch was dissolved. Nitrates were not reduced and no indol or skatol was produced from peptone. Growth occurred in Uschinsky's and Fermi's solutions but not in Cohn's solution. In all of these tests the radish organism closely resembled *Bact. campestre* and *Bact. vesicatorium*. It differed from the former in its more rapid liquefaction of gelatin, from the latter in its toleration of 4 per cent sodium chloride rather than 3 per cent, and from both in its failure to grow in Cohn's solution.

The radish organism grew well at 21°, 27°, and 32° C., and slowly at 12° C. and 35° C. The thermal death point in water suspension (6) was found to be between 50° and 52° C. Exposure for 20-, 30-, and 40-minute periods to bright sunlight greatly reduced the number of colonies in the unshaded portions of poured plates, and 60-minute exposures effected complete sterilization. The organism was not killed by a 45-day period of desiccation on glass.

TAXONOMY OF CAUSAL ORGANISM

The radish organism differs from *Bact. maculicola* McCulloch (7), the cause of a leaf-spot disease of cauliflower, and *Bact. angulatum* Fromme and Murray (1), the cause of angular leaf spot of tobacco, in that it is a yellow instead of white organism. *Bacterium tabacum* Wolf and Foster (11), the cause of tobacco wildfire, was found by Johnson, Slagg, and Murwin (5) to be pathogenic to pepper, tomato, cabbage, turnip, radish, and mustard, but also is a white rather than a yellow organism. *Bacterium melleum* Johnson causes a leaf spot of tobacco and was found pathogenic to tomato (4). However, the radish organism differs from *Bact. melleum* in being monoflagellate and in its failure to impart a honey-like color to potato-dextrose agar.

In culture the radish organism closely resembled *Bact. campestre* E.F.S. (9) but differs in host range and failure to produce vascular infection. The leaf-spotting *Bact. campestre* var. *armoraceae* McCulloch, recently described as the cause of a leaf spot of horse-radish, cabbage, and cauliflower (8), resembles the radish organism in type of lesion produced and in culture, even to a failure to grow in Cohn's solution but was reported not pathogenic to radish, mustard, or kale. Furthermore, attempts to infect horse-radish with the radish organism have been unsuccessful.

The radish organism closely resembled *Bact. vesicatorium* Doidge (3) in culture and in the type of lesion produced on tomato leaves. The lesions

formed on pepper leaves are, however, somewhat different, (Fig. 2, A and B). In parallel inoculation tests *Bact. vesicatorium* infected only tomato and pepper plants, whereas the radish organism infected radish, turnip, mustard, cauliflower, cabbage, kale, Brussels sprouts, and tobacco, in addition to tomato and pepper. In view of the fairly complete morphological and cultural similarity of these two organisms and their similar pathological effect upon tomato, it seems desirable for the present to consider the radish organism a strain of *Bact. vesicatorium*, which may be characterized as follows:

Bacterium vesicatorium raphani n. var.

Cylindrical rods, rounded at the ends, solitary, in pairs or chains; individual rods 2.0 to 3.1 μ by 0.65 to 1.2 μ; motile by a single polar flagellum, occasionally bipolar; aërobic, no spores; not conspicuously capsulate. Surface colonies on potato-dextrose agar, round, pulvinate, smooth, glistening, barium yellow, margin entire. Gelatin rapidly liquefied; no acid produced in milk; casein digested; nitrates not reduced; no acid or gas produced in media with various carbohydrates; gram-negative. Group number 211.3332513, or, according to the chart issued in 1920 by the Society of American Bacteriologists, 5322–31135–1333. Pathogenic on radish and turnip, forming lesions on leaf, petiole, cotyledon, and stem. Also pathogenic on cabbage, cauliflower, kale, Brussels sprouts, mustard, tobacco, pepper, and tomato plants and fruit.

TRANSMISSION WITH RADISH SEED

In the greenhouse work on varietal resistance, one lot of radish plants grown from seeds obtained from one firm showed infection before inoculations could be made, while seeds from two other firms, planted at the same time, produced plants with no signs of infection. Other lots of seed that had been on hand in the laboratory produced plants showing no signs of infection. Cotyledon lesions occurred abundantly on plants produced from the apparently infested lot of seeds and the causal organism was repeatedly isolated from these lesions. This evidence indicated that the organism was carried with radish seed and might be thus introduced into gardens.

More conclusive proof of seed carriage of the organism was obtained in tests with sterilized soil. In order to determine the possibility of control of the disease by seed disinfection, radish seed from seven varieties in the commercial lots found infested was immersed ten minutes in a 1–1000 solution of mercuric chloride and then planted in pots of sterilized soil. Nontreated seeds planted in similar pots served as controls. Counts were

made 28 days later to determine the percentage of infected plants in each pot.

Of the 40 plants from treated seed of the Early White Tipped variety, only 10 per cent were infected as compared with 38 per cent of the 42 plants from nontreated seed.

Of the 40 plants from treated seed of the Scarlet Globe variety, only 7 per cent were infected, while 44 per cent of the 45 plants from nontreated seed developed infection.

Of the 35 plants from treated seed of the Scarlet Turnip variety, only 8 per cent were infected. Fifty-eight per cent of the 43 plants from nontreated seed showed the disease.

The 40 plants from treated seed of the Early Giant Crimson variety showed 2 per cent of infection. Of the 40 plants grown from nontreated seed 17 per cent were infected.

Of the 36 plants from treated seed of the Woods Frame variety, only 14 per cent were infected, while 60 per cent of the 45 plants from nontreated seed developed infection.

The reduction of infection by seed treatment was still less in the two remaining varieties. Of the 38 plants from treated seed of the Early Round Red variety, 42 per cent were infected, while 62 per cent of the 40 plants from nontreated seed became diseased. Of the 50 plants from treated seed of Felton's White Box variety, 30 per cent were infected compared with 70 per cent of the 193 plants from nontreated seed.

The high incidence of infection occurring in the sterilized soil proved the disease to be seed-borne. Surface disinfection of the seed reduced the amount of infection but did not give a perfect control of the disease. Apparently, the organisms are so situated in the seed that not all can be killed by surface disinfection.

SUMMARY

The bacterial-spot disease of radish and turnip is characterized by black or brown circular lesions on the leaves and elongated, deep-sunken, black lesions on the petioles and stems of young plants.

The causal organism is a yellow monoflagellate bacterium, herein described as *Bacterium vesicatorium raphani* n. var.

While the disease is most serious on radish and turnip, particularly that following petiole infection, the organism is pathogenic also to cabbage, cauliflower, Brussels sprouts, kale, mustard, tomato, pepper, and tobacco. Tomato fruits also were infected.

On cabbage, kale, cauliflower, and Brussels sprouts the organism forms circular, chalky-white lesions on the leaves.

In inoculation tests a number of varieties of radish, turnip, Brussels sprouts, mustard, cauliflower, and cabbage were found susceptible and no resistant varieties were found.

The radish organism was compared with *Bact. campestre* and *Bact. vesicatorium* in cultural and inoculation tests. In culture the radish organism closely resembles *Bact. campestre, Bact. vesicatorium,* and, apparently, *Bact. campestre* var. *armoraceae.*

The radish organism differs from *Bact. campestre* in its wider host range and in type of symptom produced and from *Bact. campestre* var. *armoraceae* in its wider host range.

The radish organism differs from *Bact. vesicatorium* in its wider host range and type of lesion produced on pepper.

The organism was found to be carried on commercial radish seed with consequent seedling infection. Surface disinfection of seed afforded only a partial control.

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INOCULATION OF WHEAT WITH TILLETIA LEVIS (KÜHN)

E. W. Bodine and L. W. Durrell

Contrary to our usual conception of smut inoculation and infection, several investigators in the past have induced infection in cereals with different species of smut in ways differing from the normal.

As early as 1896 Hitchcock and Norton (4) used a pointed glass tube to inject a spore suspension of corn smut (*Ustilago zeae*) into different parts of corn plants and since that time this method of inoculating corn has frequently been used. Platz (7) succeeded in infecting corn plants by spraying them with a sporidial suspension and Griffiths (3) produced artificial infection of corn with conidial suspensions. The plants were inoculated either by injecting the conidial suspensions into the young parts with a hypodermic syringe or by pouring the suspension into the tops of the plants before they tasseled. Zehner (9), studying loose smut of barley (*U. nuda*), injected spore suspensions through the folded leaves and into or near the apical region of tiller growth, thereby producing smutted heads. Faris and Reed (2) produced loose kernel smut of sorghum (*Sphacelotheca cruenta*) in different parts of the plant by injection, though the only previously known means of infection by this organism was seedling penetration. Milan (5, 6), in studies with stinking smut (*Tilletia levis*), found that infection could be produced in plants past the susceptible period by puncturing the base of the culm with a needle and immersing the plant in a suspension of germinating chlamydospores.

The above results illustrate the prolonged susceptibility of the grain plants to smut infection. This also suggests that in the case of *T. levis* infection in the wheat plant might be produced after the seedling stage by introduction of inoculum.

In the following discussion the culturing of *T. levis* is described, together with an account of the use of both cultures and suspensions of spores in producing infection in plants beyond the seedling stage.

MATERIALS AND METHODS

A pure culture of *T. levis* secured from selected smut balls was first used. These smut balls were from local collections on the Experiment Station Farm, and, later, eleven other strains were cultured from smutted wheat from widely separated regions in the United States.

The smut balls were surface sterilized by dipping them into mercuric chloride 1 : 1000. They were removed and held with sterile tweezers, and with a sterile needle a small hole was carefully made in the smut ball and

spores quickly secured from the inside of the ball. These spores were then shaken onto nonnutrient agar in a petri dish. After five days the colonies resulting from germinating spores were transferred to nutrient agar and placed at room temperature for two to three days to bring out possible contamination. The cultures were then held at the more optimum temperatures of 12° to 15° C.

The fungus grows readily on several media and particularly well on Thaxter's potato hard agar. Growth is very slow at first, but in three to six weeks agar slants become well covered with a thin, snow-white, more or less prostrate, felty growth. When young, the colony is very white and smooth, with no indication of fluffiness or tufts of growth. As it gets older great masses of brown spores resembling chlamydospores are formed, giving the colony a brownish or tan color. This development or change of color occurs very rapidly in the light and at higher temperatures. This change also is accompanied by the development of the characteristic bunt odor. Microscopic examination shows the growth to consist of tangled masses of hyphae several hundred microns long, continuously producing masses of sporidia. The growth, however, is not so much by direct hyphal spread as by sporidial discharge. The sporidia are shot out from the main mass as described by Buller and Vanterpool (1) in the case of germinating chlamydospores. A veritable barrage of sporidia is discharged from the colony. These sporidia germinate on the agar where they light, producing small new colonies that repeat the process. Many sporidia are shot as far as the glass walls of the culture tube, where they stick.

A pure culture of *T. levis* is shown in figure 1, A, which illustrates the drifted manner of growth. It appears to differ materially from that figured and described by Sartoris (8).

Multisporidial cultures can easily be made from a culture with shooting sporidia by joining the flamed mouth of a tube containing a culture of *T. levis* with the flamed mouth of a flask of medium and wrapping the juncture in sterile cotton (Fig. 1, B). If left in this position two to four days sporidia are shot down to the medium below and there produce profuse colonies. This process can be repeated for weeks in making new cultures. These small white colonies can be used for inoculation or allowed to grow into a mat from which heavy suspensions of sporidia may be obtained for inoculation purposes.

Defiance wheat was used in infection tests. The seed was heavily treated with copper carbonate before planting in the greenhouse. This sterilization was effective, since no smutted plants appeared among several thousand check plants. Growth and maturity of the wheat plants were hastened by artificial light. The plants were grown in 5-foot rows and every other row was used as a check.

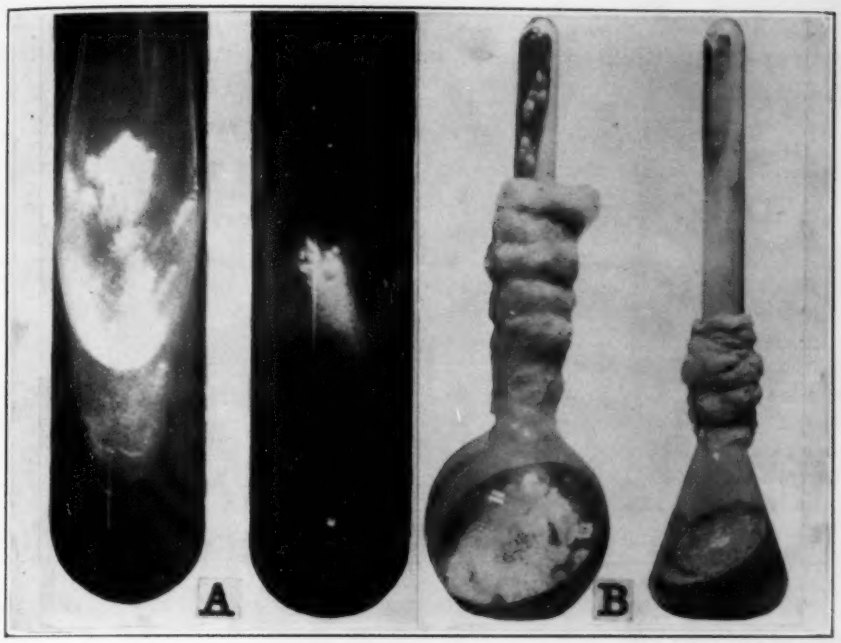

FIG. 1. A. Pure cultures of *Tilletia levis* on potato hard agar, showing the drifted character of growth by spore discharge. From material collected locally. B. Method of transfer by sporidial discharge from agar slants. Note the large colonies in flasks resulting from the great number of sporidia fallen from the culture above.

The wheat plants were inoculated at different heights, from 3½ inches to 18 inches. Three different kinds of inoculum were used, viz, small colonies six to ten days old resulting from germinating chlamydospores, spore suspension of germinating chlamydospores, and multisporidial cultures obtained by spore discharge consisting of hyphae and sporidia. The small colonies and multisporidial cultures were inserted inside the wheat sheath with an ordinary transfer needle. The spore suspensions were injected into the plant by the hypodermic needle. All inoculum was placed as near the nodes or the young growing wheat head as possible. Where suspensions were used chances of infection were probably greater because the one cubic centimeter of injected suspension filled the sheath and distributed the inoculum to the susceptible tissue.

INFECTION RESULTS

The above methods were used to inoculate 236 wheat plants in the greenhouse. For checks 322 other plants were used and, except for the inoculum, were treated as were the inoculated plants.

In the following table is a summary of the results obtained from inoculations of plants of different ages by inserting small cultures of *T. levis* into the sheath.

TABLE 1.—*Infection of wheat with Tilletia levis (Kühn)*

Height of wheat plants	Age when inoculated	Total number of plants inoculated	Percentage of smut
3½ inches	11 days	51	9.8
Control	11	75	0.0
6 inches	19	50	6.0
Control	19	50	0.0
8 inches	25	46	8.7
Control	25	65	0.0
12 inches	31	48	6.3
Control	31	71	0.0
18 inches	59	41	7.3
Control	59	61	0.0

Though the percentage of infection in the plants tested is not great, the results indicate that infection is possible by means of introducing colonies of *T. levis* in contact with the growing tissues.

The plants infected produced smutted heads many of which were distorted as in figure 2. Some of the inoculated plants showed similar distortion without visible signs of smut, though care was taken not to injure the growing stems when making inoculations.

Similar experiments also were made on plants 30 days old, 12 inches tall, grown from sterilized seed in sterilized soil. They were inoculated in three ways, viz, with small colonies, with a suspension of germinating chlamydospores, and with masses of sporidia and hyphae. The plants were held at 15° C. Only a few plants were inoculated but the results showed approximately 50 per cent infection. Adequate controls showed no infection. Inoculation of the flower and nearly mature head has failed so far. No boils so far have been produced on nodes or stems as reported for similar inoculation with *U. zeae*.

As might be expected, temperature plays a part in infection. In the first inoculation tests the temperature of the greenhouse was too high, being 20° to 25° C. Tests on a few plants held at 15° C. during the infection period showed a much higher percentage of infection. The temperature chambers were small and could only hold a few plants of wheat. A series including 15°, 20°, 25°, and 30° C. was tried with checks at 15° C. Three out of four of the plants inoculated with sporidial cultures produced

FIG. 2. Smutted heads of wheat produced by inoculation with cultures and spore suspensions. Dwarfing and distortion usually accompany smutting.

smutted heads at 15° C. and one out of three in those held at 20° C. Above these temperatures no infection was produced.

The reaction of inoculated wheat to temperature corresponds in a way to the behavior of the cultures at higher temperatures. Above 20° C. the organism in culture turns brown and produces spores which to all appearances are like the chlamydospores. At 25° and 30° C. the mycelium in culture breaks up and disintegrates.

The cultures *in vitro* follow about the same time schedule as does the fungus in the wheat plant, germinating, making vegetative growth, and darkening, with the production of brown spores in 50 to 70 days.

The wheat tissue appears susceptible to infection up to the time of flowering, though infection occurs more readily on the younger plants.

THE STATE AGRICULTURAL COLLEGE OF COLORADO,
FORT COLLINS, COLO.

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FIELD OBSERVATIONS ON STRAWBERRY DWARF IN NORTH CAROLINA

NEIL E. STEVENS AND PAUL V. MOOK

The disease of strawberries discussed in the present paper has been well described by Plakidas[1] and by Brooks.[2] It is the most conspicuous malformation of strawberry plants in the Southern States, during the summer months, and has long been recognized by growers. In Florida the disease usually has been known as "crimps," but, in Louisiana and North Carolina, it is almost always called "dwarf." The name dwarf will be used in this paper both because it was used in connection with the first published description of the disease in this country and because it has the more general usage.

Brooks proved that the disease is due to a nematode. On the basis of examinations made of diseased plants submitted by Dr. A. N. Brooks from Florida, by Dr. A. G. Plakidas from Louisiana, and by Dr. G. M. Darrow and the writers from North Carolina and other Southern States, and after an investigation of the strawberry situation in and near Plant City, Fla., in March, 1929, Dr. G. Steiner of the Office of Nematology and Technology, Bureau of Plant Industry, concluded that the causal nema is *Aphelenchus fragariae* or a very closely related form. In view of the present importance of dwarf, it seems probable that the recent field observations in North Carolina may be of use to other investigators.

THE INTRODUCTION OF DWARF INTO NEW FIELDS

The desirability of new land for strawberry culture has long been recognized by growers. For this reason, strawberries are often planted in fields freshly cleared of timber. In such fields, even though well isolated from other cultivated fields, strawberries often show a high percentage of dwarf. One such field of the Klondike variety, near Chadbourn, N. C., showed well over 90 per cent of dwarf, while an adjacent field of strawberries of the same variety, separated only by a drainage ditch but planted with a different lot of plants, showed no evidence of the disease. The only reasonable conclusion is that the disease was carried to the fields in the strawberry plants themselves.

The following experiment was designed to test the importance of this means of distribution: A field at Chadbourn, N. C., was planted in February and March, 1929, with six lots of Klondike strawberries from widely separated sources. Examination for dwarf in October, 1929, showed the

[1] Plakidas, A. G. Strawberry dwarf. Phytopath. 18: 439–444. 1928.

[2] Brooks, A. N., J. R. Watson, and Harold Mowry. Strawberries in Florida. Fla. Agr. Exp. Sta. Bul. 204. 1929.

following percentages of the disease: Plot 1–0; Plot 2–0.5; Plot 3–0.3; Plot 4–0.8; Plot 5–5.0; Plot 6–5.0. It should be noted that the only lot of plants on which no dwarf was found came from a nursery north of the region where the disease has been found.

THE RATE OF SPREAD OF DWARF UNDER FIELD CONDITIONS

The results of a "census" of the dwarf plants in certain fields near Chadbourn on different dates during the summer of 1929 (given in Table 1) show the approximate abundance of the disease in that area. That a large proportion of the plants found diseased during the later inspection were already infected at the time of the earlier inspection is, of course, highly probable, and it is apparent that if roguing is to be attempted as a means of control, all but the least infected fields will have to be rogued more than once each season.

TABLE 1.—*Number of strawberry plants showing the dwarf disease in fields near Chadbourn, North Carolina, in 1929*

Field	Date of first inspection	Percentage of plants showing dwarf	Date of second inspection	Percentage of plants showing dwarf
1	June 24	13.8	July 29	16.6
2	June 24	15.7	July 29	13.7
3	July 2	14.0	July 30	15.9
4	June 24	10.0	July 30	15.0
5	July 6	17.4	Aug. 9	20.5
6	July 18	16.8	Sept. 9	23.0
7	Aug. 6	(about 15 per cent rogued out)	Sept. 9	7.5

INFECTION OF BLAKEMORE VARIETY AT WILLARD, N. C.

Attention has already been called[3] to the fact that this disease is spread by water. The circumstances surrounding the infection of the Blakemore variety at the Coastal Plain Test Farm at Willard, N. C., point so directly to the transmission of the disease by water that they will be given in some detail. Blakemore is the result of a cross between Missionary and Howard 17. It was developed by G. M. Darrow and G. F. Waldo of the Bureau of Plant Industry[4] at the U. S. Field Station at Glenn Dale, Maryland. Not only has this variety never been found infected with dwarf at Glenn Dale, but no evidence of the disease has ever been found there on any of hundreds of varieties in the extensive breeding plots. In addition to frequent inspections by the men in charge of the breeding work and by others

[3] *Op. cit.*

[4] Darrow, Geo. M., and Geo. F. Waldo. The Blakemore strawberry. U. S. Dept. of Agr. Circular 93. 1929.

interested in strawberries, the plots at Glenn Dale have been frequently surveyed by those most interested in this disease. Brooks spent several weeks there during the summer of 1926 and made a survey of the area in August, 1929. Plakidas visited these plots in the summer of 1928 and several times in 1929. It may then be safely assumed that the Blakemore plants were free from dwarf when sent to North Carolina.

The important information regarding the various fields of Blakemore at or near Willard is given in table 2.

TABLE 2.—*Date of planting, source of plants, and condition with reference to dwarf disease of the various fields of Blakemore at or near Willard, N. C.*

Field	When planted	Condition August 4, 1929	Condition October 16, 1929	Source of plants
A	Apr., 1927	No plants	No plants	Glenn Dale, Md.
B	June, 1928	Not examined	Apparently free	Field A
C	May, 1929	Free	" "	Field A
D	June, 1928	Dwarf present	Dwarf present	Field A
E	May, 1929	Dwarf present	Dwarf present (well scattered)	Field D
F	Mar., 1929	Free	Dwarf present in the lowest portion of the field	Glenn Dale, Md.

Field A—Well drained; land next to the grapes.
Field B—A field at Teachy (about 7 miles from Willard).
Field C—Well drained; land next to the grapes.
Field D—Low land next to railroad.
Field E, Field F—Part of a higher, but less perfectly drained, field than A or C.

It will be noted that the only fields found infected in August were D and the one (E) planted directly from it but that F also developed dwarf plants before October.

The location and history of these fields are significant. Field D, one of the lowest on the farm, is located next to a railroad embankment, bordering a small brook that drains all that section of the farm; E and F are really adjacent portions of a single field, higher and better drained than D; but F lies below E and there is a low place near the center of F through which the run-off from field E drained and where during the summer of 1929 water often stood. Dwarf plants are present only in the lower part of D, are scattered more or less irregularly over E, and are confined chiefly to the lower middle portion of F.

August and September, 1928, were unusually rainy in eastern North Carolina. Rainfall for September for the entire State was the heaviest since 1908; the combined rainfall for August and September, 1928, was greater than any recorded in the State. The August rainfall for Willard was 6.89 inches and for Wilmington 7.11 inches. The September rainfall for Willard was 9.02 inches and 13.41 inches for Wilmington. This cul-

minated in the tropical storm which swept over the eastern part of the State September 18 and 19, 1928, with a total of 4.40 inches for Willard and 4.76 inches for Wilmington.

Following these excessive rains, all the lower portion of field D was submerged for several days, as was a large part of the area just across the brook that was planted with seedling strawberries from Glenn Dale, reference to which will be made later. It is this submerged portion of field D which contains the plants now infected with dwarf. The only reasonable explanation is that the dwarf plants here were infected during the submerged period in 1928, that some infected plants were transplanted to field E, thus becoming scattered over the field, and that, subsequently, the lower portion of field F became infected from these plants.

That dwarf really is an infectious disease and not a condition due to poor drainage is indicated by the history of the seedlings mentioned above. These were so badly submerged by the storms of 1928 that most of them died and the survivors were subsequently planted on higher ground where certain patches showed in October, 1929, the most severe cases of dwarf that the writers have ever seen.

PROBABLE SOURCE OF THE INFECTION ON THE BLAKEMORE VARIETY AT WILLARD

Strawberries have been grown commercially at several places on the test farm at Willard, many of which were no doubt diseased. Throughout the seasons of 1928 and 1929, a field of about two acres of the Missionary variety, showing two to five per cent infection with dwarf, was grown on the test farm on an area that drains directly into the brook running beside field D. The planting stock for this field was obtained from an adjoining area, plowed up after fruiting in the spring of 1928. Fields A, C, E, and F do not drain toward D. The only apparent source of infection was the two-acre field of the Missionary variety from which water spread the disease to field D.

SUMMARY

Field observations in North Carolina indicate that the dwarf disease of strawberries is carried to new land by the planting of infected plants.

Typical fields of the Klondike variety near Chadbourn showed from 10 to 23 per cent of this disease during the summer of 1929. Several fields showed as high as 90 per cent.

Plants of the Blakemore variety originated at Glenn Dale, Md., which had never shown the disease, became infected during 1928 and 1929 at Willard, N. C.

The circumstances indicate that the disease was spread by water.

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EPHELIS-LIKE CONIDIA AND FLORET STERILITY IN ARISTIDA

WILLIAM W. DIEHL[1]

An abnormal specimen of *Aristida glauca*, gathered by B. C. Tharp in Lampasas County, Texas, in 1924, showed certain features in comparison with accompanying normal material which appear not to be recorded. This account deals with a short discussion of the abnormality and of the Ephelis-like fungus evident microscopically within the diseased spikelets of the grass.

THE DISEASED HOST PLANT

The diseased and normal plants are so unlike that it is difficult to recognize them as of the same species (Fig. 1, A). Grasses infected by species of Ephelis usually show dwarfing, but rarely so extreme as in this instance.

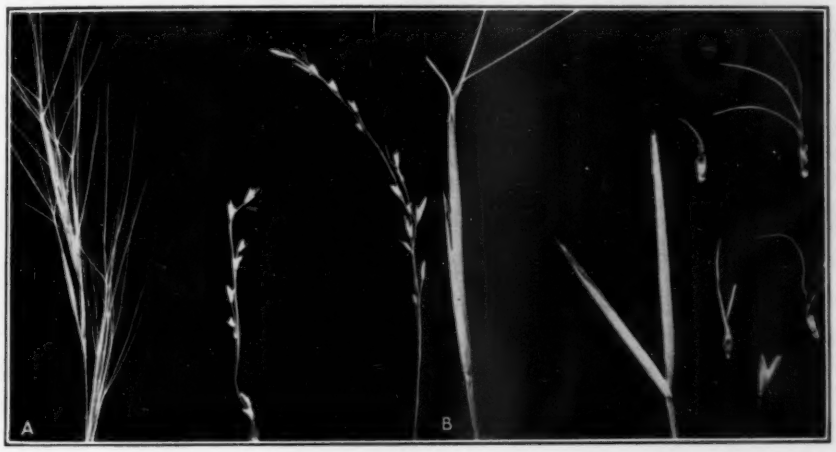

FIG. 1. A. (×1.2.) Normal inflorescence at left; diseased inflorescence at right. B. (×2.2.) Spikelets of Aristida: Left, normal spikelet; center, normal glumes, floret removed; right, diseased spikelets, at bottom, glumes only, floret removed.

In certain species of Ephelis the fungus surrounds the undeveloped inflorescence of the host which it encases, preventing thereby further floral development. In this instance, however, the diseased specimen is distinguishable superficially only by a consistent dwarfing of the entire inflorescence. Individual spikelets compared with normal ones (Fig. 1, B) are uniformly dwarfed. The glumes are found constantly to be approximately

[1] Thanks are due to Mrs. Agnes Chase and Dr. A. S. Hitchcock for verification of the grass host and to Mr. J. F. Brewer for the photographs.

one-sixth the length of normal glumes, although nearly as broad. The florets, which in Aristida are but one to the spikelet, are reduced in size proportionately. The grains failed to develop whether the coincident fungus mass was slight or extensive. A section (Fig. 2, A) through a

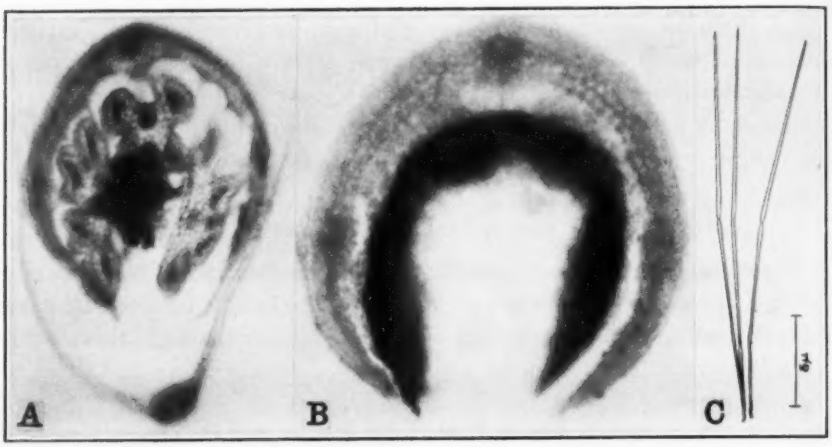

FIG. 2. A. (×78.) Free-hand section of diseased spikelet transverse to the main axis, showing inner glume and lemma enclosing stamens and undeveloped ovary with small central fungus mass. B. (×120.) Cross section—as in A—of a mature sclerotium, showing lemma partially enclosing the fungus layer with inner cavity lined by a mass of conidia. C. Interpretative drawing of conidia and conidiophores.

sterile spikelet with but slight fungous development shows little displacement of host tissues, although other sections (Fig. 2, B) where the fungous growth had developed extensively showed extreme displacement of host by fungous tissue to the extent of entire elimination of all the floret except the lemma.

THE FUNGUS

The fungus mass found in sterile florets may be regarded as sclerotioid, even though certain host tissues are included; this is homologous with the structure found in *Balansia hypoxylon* (*Ephelis borealis* E. & E.) and described by Atkinson[2] as a "pseudosclerotium." Mature sclerotioid masses where the floret tissues were largely displaced (Fig. 2, B), the lemma remaining as a rind, are quite variable in size, but none were observed over 2 mm. in length; they also vary in color from gray through brown to black. These mature bodies differ from sclerotia hitherto recognized in Ephelis in that here the entire central region is a cavity filled with a mass of acicular

[2] Atkinson, G. F., in The Genera Balansia, etc., p. 249. Jour. Mycol. 11: 248–267. 1905.

conidia of the type found in a normal Ephelis fructification. These conidia are of a dark greenish cast when in mass, but hyaline when single, 20–23 × 1–2 μ, rarely up to 29 μ long, borne upon short conidiophores not exceeding 3 × 1 μ. Although not clearly demonstrated, separation in suggested by the regular constriction and by frequent division into three segments. The protoplasmic content of these conidia is definitely guttulate (Fig. 2, C). The layer of spores presents the appearance of a palisade lining the lemma. They readily become detached from the conidiophores, fill the cavity, and extrude through the vertical aperture along the side not covered by the lemma.

The extreme variability in size and state of maturity of the fungus mass within the florets suggests a possible explanation for some obscure instances of floret sterility in other grasses. There should be no confusion, however, between this type of dwarfing and that dwarfing and malformation characteristic in Aristida when infected by species of Sorosporium. It may be significant in this connection to note that the writer's various attempts to culture living material of Ephelis on artificial media have been uniformly unsuccessful.[3]

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WASHINGTON, D. C.

[3] After the above account had been sent to press the writer accompanied by Professor Tharp of Austin, Texas, visited the locality in Lampasas County on June 4, 1930, where these characteristically diseased plants are numerous. The writer is very grateful to Professor Tharp for transportation and other courtesies in this connection.

ASTEROCYSTIS RADICIS IN THE ROOTS OF CEREALS IN SASKATCHEWAN

T. C. VANTERPOOL

While conducting a systematic microscopic examination of the root systems of wheat, oat, barley, and rye seedlings grown in pots of soil collected from widely distributed localities of Saskatchewan, the writer was struck by the frequent occurrence of sporangia and hypnospores of *Asterocystis radicis* de Wild. (*Olpidium radicicolum* de Wild. (1) in the finer roots of oats and their occasional presence in wheat, barley, and rye. This fungus was observed also in roots of maize, western rye grass (*Agropyron tenerum* Vasey), and field mustard (*Sinapis arvensis* L.) seedlings in potted soil and in barley seedlings from the field. The pots were kept well watered throughout the experiments. As a rule, infection was slight and the roots appeared none the worse because of the presence of the fungus. Occasionally, however, infection of the oat roots was so intense (Fig. 1, C and D) that the affected roots showed signs of necrosis. In such instances the normal activity of the plants was doubtless greatly impaired, as evidenced by the slight yellow discoloration of the leaves of the seedlings. The most heavily infested soils appeared to be those with highest water-holding capacity.

In the light of the contributions of European investigators and more especially of the recent work of Guyot (4), these observations take on a new interest and perhaps warrant this note. According to this author de Wildeman reported as early as 1893 the presence of *A. radicis* in the roots of Gramineae. And Marchal observed this fungus on oats, in 1901, but never found it on wheat in the course of his experiments. More recently, other Continental workers (3, 5, 8) have observed its occurrence in oats and wheat. Rives (8), in 1925, attributes to this fungus the cause of an occasional root-scorch disease of oats in France and believes that the ability of the fungus to attack depends upon conditions unfavorable to the plant. Fron and Gaillat (3) were able to infect oats and several grasses with it but did not get infection on wheat. Guyot (4) could readily obtain infection of oat roots; but only in very heavily infested soil could he infect wheat. In the spring of 1926, he found *A. radicis* and other fungi associated with the roots of chlorotic and yellowed winter-wheat and oat plants obtained from low, wet spots in fields near Paris. Unlike Rives, he is inclined to believe that the scorch and chlorosis of these plants are not caused by *A. radicis* but are due to physiological disturbances brought about by excessive moisture. Peyronel (5, 6, 7) has frequently encountered

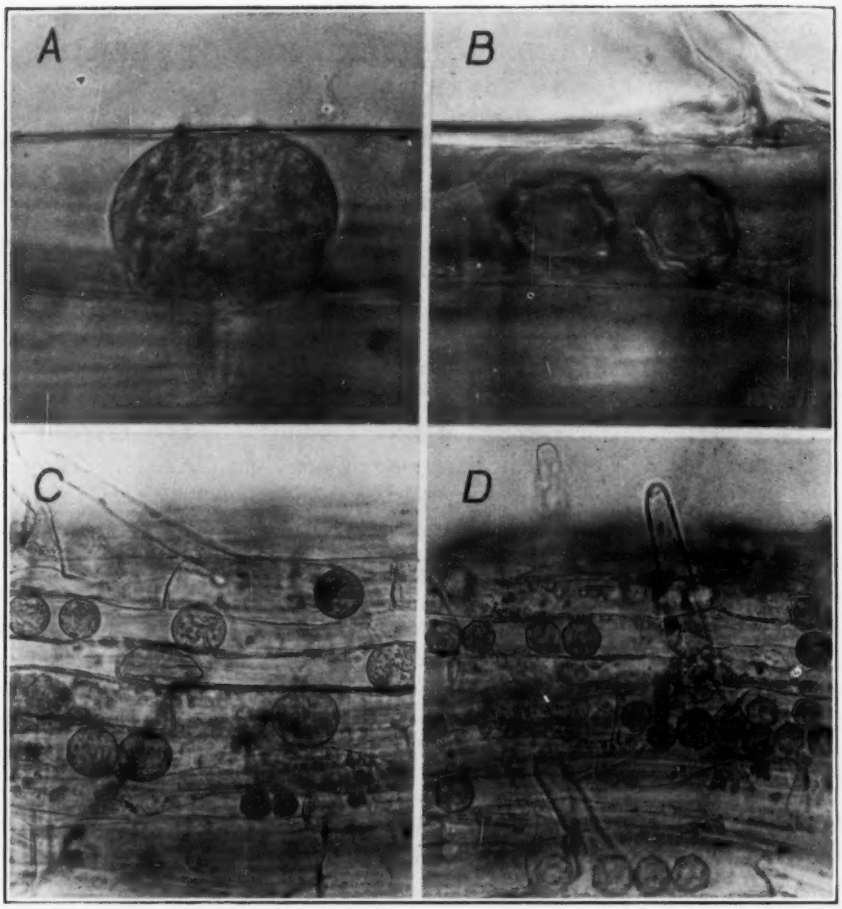

FIG. 1. *Asterocystis radicis.* A. Sporangium in an epidermal cell of an oat root with two very short exit tubes protruding slightly through the outer cell wall of the host. Stained with lacto-phenol-cotton blue. ×900. B. Two hypnospores in an epidermal cell of a wheat root. Photographed *in vivo.* ×900. C. Surface view of sporangia in an oat root. An empty sporangial sac is to be seen in the center of the photograph. Stained with lacto-phenol-acid fuchsin. ×280. D. Surface view of a portion of an oat root showing hypnospores in process of formation from sporangia (zygotes) and rounded-up zoöspores within the host tissue. Stained with lacto-phenol-acid fuchsin. ×280.

A. radicis on both healthy and decayed wheat roots while investigating mycorrhiza in that cereal, and he is of the belief that this fungus can become a virulent parasite under conditions unfavorable to the host. Guyot, in his recent contribution to the taxonomy and biology of *A. radicis*, contends that the characters hitherto used to separate *A. radicis* and *Olpidium bras-*

sicae (Wor.) Dangeard are no longer tenable and he strongly doubts whether the two forms can be maintained as distinct species. An exit tube for the discharge of the zoöspores from the sporangium was previously supposed to be lacking in *A. radicis*, but Guyot pointed out that the sporangia of *A. radicis* in oats and in *Poa annua* frequently possess one or more exit tubes. *A. radicis* has not been reported from America, but Guyot is of the opinion that the fungus described by Bensuade (2), in 1923, in the roots of tomato, tobacco, and cabbage in Wisconsin as *O. brassicae* is none other than *A. radicis*.

During his studies the present writer has frequently observed long exit tubes in the mature sporangia in oats, but less commonly in wheat. As is characteristic of *A. radicis*, the sporangia and hypnospores were found singly or in aggregates in the epidermal, root hair, and outermost cortical cells of the root (Fig. 1). The hypnospores varied in size from 8 μ to 40 μ in diameter, with an average of 18 μ (Fig. 1, B and D). The sporangia averaged slightly larger (Fig. 1, A and C). Occasionally, zoöspores which had been discharged internally would be seen in active motion confined in a host cell. The zoöspores were uniflagellate with the flagellum equal in length to about six times the diameter of the spore. Penetration of a root hair by a zoöspore was observed. (Fig. 2B) The zoöspore, however, is able to penetrate an epidermal cell of the root directly (*cf.* 9), as evidenced by the frequent presence of sporangia and hypnospores in epidermal cells just behind the root tip in the region where root hairs had not yet developed. Cells of the root cap also are sometimes invaded. The

FIG. 2. A. *Asterocystis radicis*. At the top: An empty sporangial sac with papilla-like exit tube showing at the surface as a flattened ring. Below: Two immature individuals. ×900. B. *Asterocystis radicis*. A zoöspore penetrating a root hair of a wheat seedling grown in sterile water in a small flask. ×1350.

fungus shows up well in the host tissue when stained with lacto-phenol-cotton blue or lacto-phenol-acid fuchsin. Dilute Gram's iodine solution stains the sporangia bright brick red, while the hypnospores are stained a reddish brown. This reagent is also well suited for showing up the zoöspore flagellum.

It appears from the pot experiments conducted in this greenhouse that *A. radicis* is a normal inhabitant of Saskatchewan soils. It is doubtless maintained from year to year in the roots of various mustards and grasses as well as on the cultivated cereals. From the writer's findings and those of European workers it seems probable that only under exceptionally favorable conditions would this fungus cause any significant damage, and then only on oats. Alone, it need hardly be regarded as of any economic importance; but, as paving the way for or aggravating the damage caused by more vigorous parasites, it may doubtless become one of the many contributing factors in the root-rot complex of oats in some localities.

PHYTOPATHOLOGICAL LABORATORY,
UNIVERSITY OF SASKATCHEWAN,
SASKATOON, CANADA.

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PHYTOPATHOLOGICAL NOTES

A celluloid cell for inoculation of plants with insect vectors.—Cells of different sizes made of glass, wire mesh, and celluloid have been used by the writer in his work with tomato yellows, transmitted by a leaf hopper, *Eutettix tenellus* Baker. At first, various types of cells used by other workers were tried. Later, a modification of a celluloid cell was evolved that proved very convenient. The appearance of this cell when attached to the plant is illustrated in figure 1. It is about 5 inches in length and 2½ inches in diameter. To make such cells, celluloid sheets 50 inches long and 20 inches wide were obtained and cut, each, into 24 equal pieces, approximately 5 x 8⅓ inches in size. Glass bottles about 2½ inches in diameter were used as forms to give these cells a cylindrical shape. While holding with one hand the small piece of celluloid so as to partly envelop the bottle, a cement was applied to the edges which were to lap. There are commercial cements on the market suitable for this purpose, but a good one may be quickly and cheaply prepared by dissolving pieces of celluloid in acetone. After smearing the cement along the edges of the two opposed surfaces the latter were pressed together and the cylinder, with its form inside, was laid on the table, seam side down, with some weight on the top. When the cemented parts harden in this position, a permanent union (Fig. 1, e), as well as a convenient shape, is obtained, but if taken off the form while the seam is still soft, the cylindrical shape of the tube may be lost. A piece of cloth of a desired density was fastened to one end of the cylinder by a couple of rubber bands and the space between these bands was saturated with the celluloid cement (Fig. 1, a). When this was dry, the superfluous edges of the cloth were trimmed close to the rubber band. A small hole was made in the center of the cloth for introducing insects. This was kept plugged with cotton when not in use (Fig. 1, b). At the other end, a cloth tube 4 or 5 inches in length and of the same diameter as the celluloid tube was affixed in a manner similar to the one just described (Fig. 1, c). The portion of the plant chosen for the exposure to insects is introduced into the cell through the open cloth end, which is then tied up around the stem of the plant with a string (Fig. 1, d). This cell was used in work with *E. tenellus* and *Paratrioza cockerellii* Sulc. and was found to be very convenient. No doubt, it may be advantageously used in connection with other insect vectors. It is very light and in most cases needs no support, except of the plant stem itself. In this respect it is much preferred to all kinds of glass cells and lamp chimneys. It permits the circulation of air, prevents excessive accumulation of condensation water, and does not obstruct the entrance of light. The enclosed plant parts and the insects may be

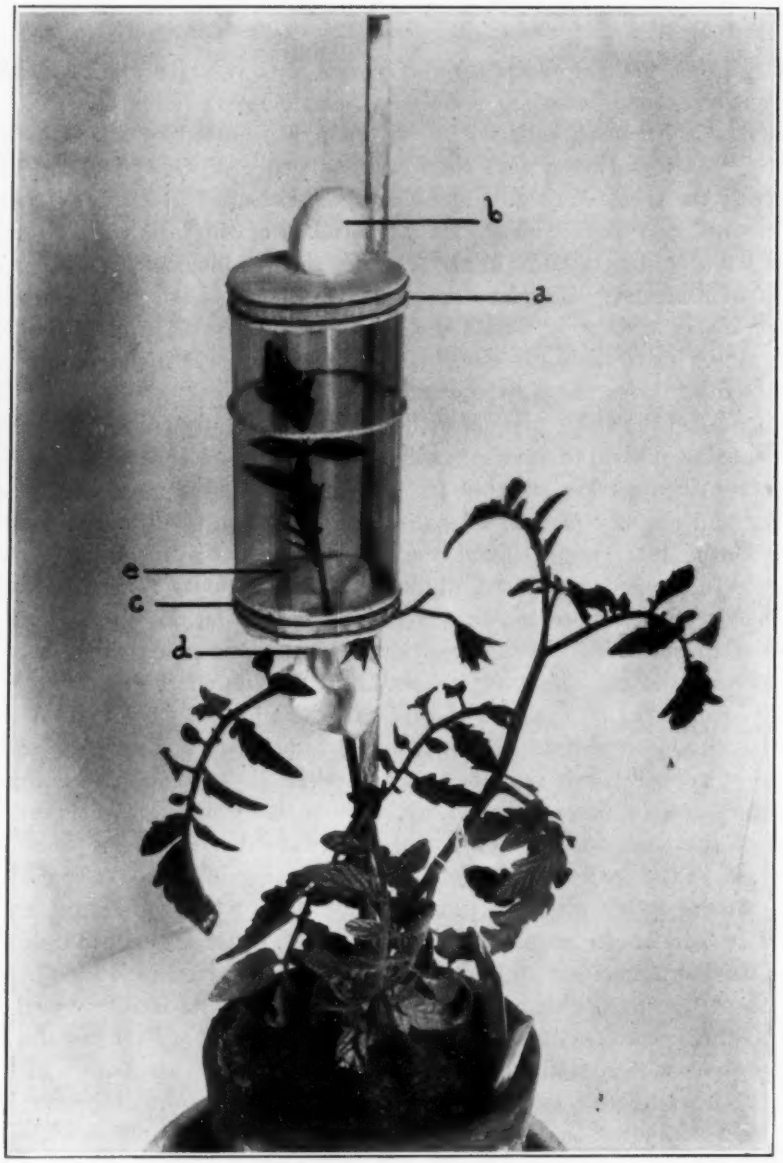

FIG. 1.—A small celluloid inoculation chamber attached to a growing tip of a tomato plant for exposure to viruliferous insects.

conveniently observed during the experiment. The insects may be readily located and removed with a suction tube. Finally, its size is such that, while giving plenty of room for the inclosure, it also allows an economic use of the material.—MICHAEL SHAPOVALOV, Western Tomato Disease Investigations, U. S. Department of Agriculture, Riverside, California.

Rhizoctonia on wild potato.—The small wild potato, *Solanum Jamesii,* occurs in the southwestern part of Colorado and adjacent regions. It produces small tubers that have been described as perfect miniature Early Ohios. Through the courtesy of Dr. Edna L. Johnson, of Boulder, the writer received a number of these tubers that bore black bodies having the appearance of sclerotia. Microscopic examination showed these black bodies to be sclerotia and later platings in all cases resulted in cultures of Rhizoctonia. The strain of Rhizoctonia obtained, however, has a growth habit differing from that of the stock laboratory cultures of *Rhizoctonia solani.*

The writer has been unable to find any reference to *Solanum Jamesii* as a host for Rhizoctonia. The occurrence of the fungus on the tubers of the little wild potato is interesting in the light of the prevalence of this organism in the soils of the State.—E. J. STARKEY, Colo. Agr. College, Ft. Collins, Colorado.

Note regarding a possible influence of soil reaction on development of powdery mildew on cowpeas.—Plant-pathological and soil-fertility workers quite often meet on common ground to study problems of mutual interest. Take, for example, powdery and common scab of potatoes, cotton wilt, and cotton rust; or, in the case of a deficiency in the soil of nitrogen, phosphorus, potassium, magnesium, manganese, iron, and other essential elements, pathological manifestations of one kind or another, either directly or indirectly, often are in evidence. The influence of soil, as to location and composition, is believed important by plant pathologists; also, the well-known effective control of certain diseases with materials like lime, or perhaps augmenting another plant trouble equally as effectively with the same treatment. The present note refers briefly to a case of relative susceptibility of cowpea plants to ordinary powdery mildew as influenced apparently by soil reaction.

During the season of 1929, an experiment was started at Arlington Experiment Farm to determine the comparative effectiveness of a number of phosphatic carriers, including superphosphate, lime or rock phosphate, soft phosphate, and dicalcium phosphate. The experiment is being conducted in an outside cage. Three plant indicators are employed, namely, wheat, cowpeas (Whippoorwill variety), and millet. These are grown in rotation in the order given. A duplicate series is included.

As it was desired to study the comparative effectiveness of the different phosphatic materials in a soil of varying reaction, a distinctly acid soil, pH 4.8, on Arlington Farm, was selected for the pot experiments. By the addition of hydrated lime, the reaction was changed to pH 6.4 and pH 7.5, respectively.

Without attempting to consider details pertaining to soil-fertility matters, attention is simply called to figures 1 and 2, which show the relative susceptibility of the cowpea plants to mildew under prevalent conditions. These specimens were taken from the nonfertilized soils. The matter is

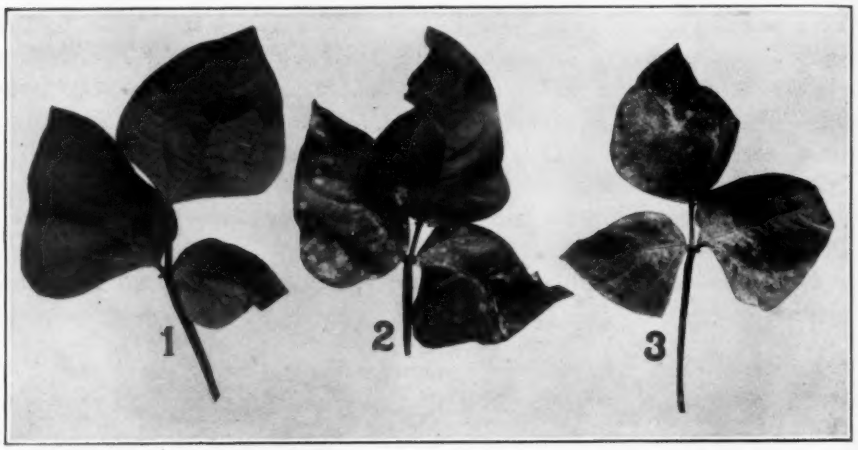

FIG. 1.—Showing possible influence of soil reaction on development of ordinary powdery mildew on cowpeas. No. 1—Soil reaction of pH 4.8. No. 2—Soil reaction of pH 6.4. No. 3—Soil reaction of pH 7.5.

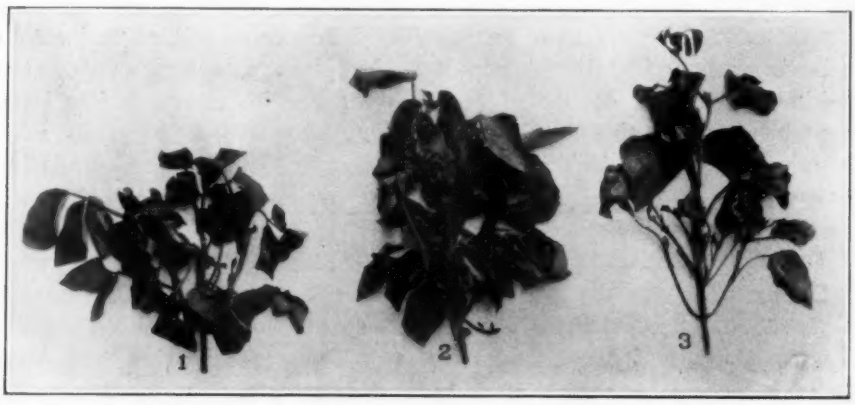

FIG. 2.—Larger specimens of cowpea material. No. 1—Soil reaction of pH 4.8. No. 2—Soil reaction of pH 6.4. No. 3—Soil reaction of pH 7.5.

recorded at this time because of the clear-cut manifestation of the disease under an alkaline-soil reaction and the equally clear-cut improvement shown with increasing soil acidity, and, furthermore, because it was felt the observation might be of interest to plant pathologists engaged in studying forage-crop diseases. In the case of cowpea plants grown on soil possessing a pH of 4.8 (No. 1), there was not evident a spot of mildew on a single leaf or stem. With a pH of 6.4 (No. 2), some mildew was found, and with a pH of 7.5 (No. 3), the ravages of the disease were quite marked, as indicated by leaves and stems.

Whether the soil acidity retarded the development of the disease through plant-sap changes, or whether the added lime may have influenced the character of the plant sap in such a way as to make it a more favorable culture medium for the organism, is not clear. No opportunity was afforded at the time to determine pH values of the respective plant saps or otherwise examine them for plant-food constituents.—B. E. Brown, Senior Biochemist, Soil Fertility Investigations, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Notes on flooding injury to strawberries.—The strawberry section of southern Alabama is located around the towns of Castleberry and Brewton in the drainage basin of the Conecuh River. In March, 1929, this basin experienced a very severe flood which was far more extensive and caused much more damage than the previous great ones of 1861 and 1916.

The last of three periods of heavy rainfall during the month of February brought the rivers to flood stage and the saturated soil afforded an excellent foundation for the flood. The climax followed the enormous rainfall of March 13 to 15. At Brewton the precipitation during the later period was 16.26 inches, making a total of 24 inches in less than a month. The crest of the flood occurred on March 15. Parts of the region were inundated to a depth of 10 or more feet. The duration of the flood varied with the topography of the land. In general, the water had receded to such an extent by March 21 that no cultivated fields were submerged. The soil remained saturated, however, for an appreciable time because of additional rainfall from March 21 to 23.

The observations here reported are a part of the study of the strawberry-disease developments subsequent to the flood. The studies were made during the second week of April, approximately four weeks after the period of maximum precipitation. The Missionary variety is by far the most popular one in the Castleberry section, although both the Klondike and Aroma varieties are grown to some extent. At the time of the flood the majority of the plantings were in full bloom, the largest berries being slightly larger than peas. At the time of the writer's observation four weeks later

the plants, in general, were in full bearing. The period of time that elapsed between full bloom and full bearing was therefore prolonged approximately a week by the unusual weather conditions.

Most of he strawberry plantings were located on the uplands and, consequently, were not submerged. The heavy driving rains had caused deep gullies in the fields and had washed out many plants by the roots. A large part of the pine-straw mulch was washed from under the plants, leaving the berries in contact with the soil. They were thus directly exposed to infection by soil fungi. By far the greatest injury to the crop, because of the wet period, consisted in decay of the berries. In some fields as much as 50 per cent of the berries on the plants were partly decayed. A small percentage of the decay appeared to be hard rot, caused by *Rhizoctonia* sp. Most of it, however, exhibited the typical symptoms of leather rot caused by *Phytophthora cactorum* (Leb. and Cohn) Schroet.

In one of the lowland fields of Missionary strawberries from which the water had drained slowly, it was possible to observe the effect on the plants of various periods of submergence. The field was located on the sloping side of a lake having no visible outlet. At various intervals as the flood waters receded the water line was marked by the owner so that he was able to tell approximately how long each part of the field had been submerged. A few of the rows in the upper part of the field had not been flooded and, at the time of the writer's observation, were in excellent bearing. The adjacent rows on slightly lower ground appeared to be the same, although some of the plants had been covered for as long as two and a half days. No stakes marking the various water levels had been placed on the area which had been covered from six to fourteen days. Apparently no plants submerged less than a week were killed. The rows covered longer than a week contained numerous dead plants. The number of plants that were killed increased in proportion to the number of days of submergence, so that, near the stake marking the level on the fourteenth day, only a few of the mother plants having stout crowns were alive. There were no signs of life in any plant that had been covered longer than two weeks.

In a neighboring field Aroma strawberries had been planted between rows of high-bush blueberries, probably *Vaccinium virgatum* Ait. Water had remained in the depressions in the field and in some of these areas had killed both the strawberries and the blueberries. The zones of injured and dead plants indicated that the blueberries had about the same degree of flood tolerance as the strawberries.—Cyril O. Bratley, Bureau of Plant Industry, U. S. Department of Agriculture.

Field observations as an aid in avoiding diseased cotton seed for planting.—It is a generally accepted fact that cotton seed produced on dis-

eased plants, particularly plants infected with boll rots, are inferior to seed produced by healthy plants. If this be true, it should be possible to predict from field observations whether or not cotton seed produced in certain areas should be saved for planting. In the latter part of the season of 1928, the lower, or eastern half of the State of South Carolina, experienced an unusual amount of rain. Conditions were ideal for the development and dissemination of disease organisms. As was expected, these factors brought about an abundance of boll rots. Observations in the upper half of the State, where better weather prevailed, gave circumstantial evidence that the seed produced in the upper part of the State would be quite superior to that of the lower part. Germination tests of both upper- and lower-State seed were made to determine the planting value of seed from these localities.

Seeds were germinated in specially constructed germination pans. The same amount by weight of sand was placed in the pans and an equal weight of water added to each. Two hundred seeds of each sample were tested, the seeds being slightly pressed into the moist sand. Germination took place at a temperature range of 25° to 30° C. In these tests, all seeds with emerging root tip were considered as germinated.

Twenty-five samples of seed from the lower part of the State and 25 samples from the upper part were tested for germination. The average germination of the entire 25 samples of lower-State seed was 51.68 per cent, as compared to a germination of 79.64 per cent in the 25 samples of upper-State seed. The difference in the two lots of seed was 27.96 ± 1.1845.

Isolations from diseased bolls from several diseased fields gave an abundance of *Diplodia gossypina* and *Glomerella gossypii*. In most instances one or more species of Fusarium were obtained from the diseased material. None of these was *F. vasinfectum*, but seedlings grown in pots inoculated with the various cultures of Fusarium obtained from cotton bolls were considerably stunted in growth, due to root injury.

Diplodia gossypina was the most common pathogene developing on the seed in the germination pans. There was also a considerable amount of *Glomerella gossypii*, *Fusarium* sp., and various kinds of bacteria. None of seven morphologically distinct bacterial cultures, isolated from diseased bolls, gave evidence of parasitism on seedling cotton. However, many seeds which failed to begin growth in the germination pans were completely covered with a bacterial ooze. The symptoms of these decayed seed were typically those of a bacterial soft rot. Due to the fact that the writer left the experiment station in June, 1929, a detailed study of the organisms was not made.

Limited pathogenic studies indicated that several species of fungi and bacteria influence the germination and ultimate development of cotton seed

and seedlings, even though some of the organisms may appear saprophytic on the mature cotton boll.

The conditions existing in South Carolina in 1928 may have been unusual and may not recur soon. However, similar conditions can be expected in certain cotton-growing sections of the South—at least occasionally. The opportunity may arise in the future for other workers to make similar observations that will warrant certain recommendations to the cotton grower regarding the source of seed. For that reason the foregoing results are here presented.—JAMES M. WALLACE, Twin Falls, Idaho.

BOOK REVIEW

Hodgman, Charles D., and A. Lange Norbert. Handbook of Chemistry and Physics. 14th ed., 1386 pp. Chemical Rubber Publishing Co.: Cleveland, Ohio, 1929. Price $5.

In America the term applied mycology has never been approved as a proper appellation for the study of plant diseases, partly because in certain industrial connections it transcends the field of plant-disease control, but chiefly because plant pathology is so much more varied a science than mycology. Though the foundations of all phases of experimental botany are laid in chemistry and physics, phytopathology, in its more practical aspects, partakes also of the nature of an engineering science, and in this respect might be said to stand in about the same relation to plant physiology as industrial and engineering chemistry do to theoretical chemistry. This is due to the fact that so large a part of the service rendered by any applied natural science is for the purpose of establishing controls and correcting tendencies that have gone wrong. If an authoritative and reasonably complete reference book to chemical and physical data is indispensable equipment to experimental biologists in general, how much more essential is it for the plant pathologist?

The series of handbooks of chemistry and physics published by the Chemical Rubber Publishing Company has long been recognized by the unprofessional chemists, physicists and mathematicians, who constitute the majority of workers in experimental biology, as one of their principal supports in working with the resources and methods of these sciences. The latest edition has been expanded in certain directions that make it even more useful to the student who is concerned with the measurement of environmental factors and the responses of organisms. In fact, most of the chemical and physical data found in such encyclopedic works as the International Critical Tables and Smithsonian Tables that are of concern to the biologist and general laboratory worker are collected here.

Of special note among the subjects the treatment of which is new or completely revised are the following: A table of indicators, with an explanation of the properties thereof and showing the name, source, and pH range; the preparation of Clark and Lubs indicator solutions; conversion factors for E.M.F. to pH with the standard electrodes; electrolytic and oxidation potentials; properties of dry and saturated air including volume changes with temperature and pressure, heat capacity. etc.; similar data for air of various percentages of saturation; tables of heat of combustion of organic compounds, gases, and various substances, including coal, wood, and oil; tables showing the functions, uses, and compositions of foods; wave-length of prin-

cipal lines in the spectra of the elements; transmission of radiation by different neutral and colored glass; physical constants of resins and gums in addition to those of oils, fats, waxes, and inorganic and organic compounds generally; interest tables; a synopsis of each of the elements; examples of various volumetric reactions with gram equivalents.

The tabulated data of many other subjects too numerous to mention appear as in previous editions. Of special interest in the present relation are: Preparation and standardization of laboratory reagents, solubility of cane sugar, gases, and inorganic compounds in water, cuprous oxide equivalents of various sugars, hydrometer and thermometer conversion tables, vapor tension of water, psychrometric tables, photometric standards, photographic formulae, various weights and measures. An index of over 1000 entries affords easy accessibility to the diverse information given.—F. WEISS, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.